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EFFECTS OF *beta*-RADIATING ISOTOPES IN FRUIT TREES

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SINCE 1944 experiments with mutation-inducing agents have been performed in fruit trees at the Balsgård Fruit Breeding Institute (GRANHALL, GUSTAFSSON, NILSSON, and OLDÉN 1949; GRANHALL, 1949, 1951). In several respects the genera *Malus*, *Pyrus* and *Prunus* present themselves as an interesting perennial material for theoretical mutation studies and for practical mutation-breeding experiments. They are, as a rule, characterized by a markedly heterozygous constitution which forms a good background for the detecting of vegetative hereditary alterations, at least in the lower ploidy stages. Mutations obtained can be perpetuated and propagated by means of grafting and budding. From the breeder's point of view somatic mutations in sufficient frequency would offer a quicker way of procuring new material for selection than the usual cross-breeding followed by slow seedling development till fruit-bearing age. As to cytological studies, the fruit trees are, however, comparatively unagreeable to handle.

During 1944—48 only X-rays were used at the treatment of apples, subsequently also of pears and cherries. Results obtained with other agents in cereals, especially in barley (GUSTAFSSON and MAC KEY, 1948; EHRENBERG, GUSTAFSSON, LEVAN, and VON WETTSTEIN, 1949), gave, however, rise to a widening of the fruit tree experiments. In 1949 neutrons and radiophosphorus were thus included; in 1950 also radiosulphur.

The present paper will give a description of the technique employed at the application of the two β-radiating isotopes P32 and S35 and also a comparison between their primary biological effects and those deriving from X-ray and neutron treatment in the fruit trees.

The investigations have been carried out in cooperation with Prof. ÅKE GUSTAFSSON, Stockholm, and have been defrayed by grants from the Knut and Alice Wallenberg Foundation. The authors also give their acknowledgements to Amanuensis MARGARETA BRUNNBERG, Stockholm, for her valuable help with the autoradiographs, and to Assistant K. A.

ANJOU, Balsgård, who has performed many of the field observations of the material and has also drawn the pictures of the injected trees.

THE IONIZATION PROPERTIES OF THE DIFFERENT AGENTS.

X-rays (in our case unfiltered, obtained from a Coolidge-tube, which was run at 175 kV) interact with matter through the projection of fast electrons, which are slowed down as they give energy for the ionization of molecules which they pass. At each ionization a pair of ions, one positive and one negative, is formed. Especially the positive ions are liable to chemical recombination or reaction.

It can be expected *a priori* that different kinds of ionizing radiations may have a differential action, especially due to different properties in the following respects (*cf.* GUSTAFSSON, EHRENBERG, and BRUNNBERG, 1950):

(1) The *ionization density* (also called specific ionization, ion pairs per μ of tissue) may be an important factor, as some produced changes, especially in chromosomes (*cf.* LEA, 1946), seem to be due to several ionizations within a certain small volume, whereas others are due to one single ionization. The first type of changes is obviously promoted by densely ionizing radiations, and *vice versa* (*cf.* EHRENBERG, GUSTAFSSON, and NYBOM, 1952).

(2) *The distribution of the radiation within the tissue* can be varied by aid of radioactive isotopes, the chemical nature of which determines on what parts of the irradiated object the radiation is concentrated. In this connection a short *range* of the radiation will increase its concentrating on a certain part of the object.

(3) It is important to vary the *duration of irradiation*, especially in order to ensure a covering of both radiation-sensitive as well as radiation-insensitive periods of the investigated plants.

Especially as regards points (2) and (3) above, the radioactive isotopes have given us useful means to vary the irradiation conditions in desired directions. Also a variation in ionization density may be achieved by the use of the β-radiating isotopes P32 and S35, which both project faster electrons than do X-ray quanta. Their specific ionizations are 8 and 51 ion pairs per μ of tissue (of unit density), in contrast to the 100 ion pairs per μ of tissue produced by X-rays.

Fast neutrons (which principally interact with living matter through projection of protons) and α-rays (from polonium or radon) give rise

to still higher ion densities, *i. e.* 800 and 4000 ion pairs/μ, respectively. (For these and other physical properties of radiations, see Table 1 of GUSTAFSSON *et al.*, 1950; also EHRENBERG and NYBOM, 1952.)

APPLICATION, MEASUREMENT, AND UPTAKE OF THE ISOTOPES.

In all experiments with the heavy phosphorus isotope, P32, it has been obtained as phosphoric acid, H_3PO_4, in water solution: in 1949 from Oak Ridge, U. S. A., and in later years from the Harwell pile in England. As a rule it has been carrier-free, but in some cases it has contained 1—1,5 μ-mol of inactive H_3PO_4 per mC of P32. This small amount of carrier will probably have but a slight decreasing effect on the uptake rate, compared to a higher concentration (*cf.* EHRENBERG *et al.*, 1949). On the other hand, the presence of a small amount of carrier is advisable in practical work as it diminishes the adsorption of active phosphate on glass ware and other instruments.

The radiosulphur was exclusively obtained from Harwell, as H_2SO_4. Carrier-free solutions were used in order to obtain highest possible uptake rate. In addition, all S35 solutions have contained a certain amount of HCl (up to 0,1 mmol/ml), which, owing to the method of production, could not be totally avoided. Before use, all isotope solutions were neutralized with NaOH to a pH between 6 and 7, thus brought to contain chiefly, in the case of P32, the ions $H_2PO_4^-$ and HPO_4^{2-}, and, in the case of S35, the sulphate and inactive chloride ions.

The analytical activity determinations were performed with a Geiger-Müller counter with a tube provided with a thin mica window, penetrable to the low energy β-particles from S35. As to methods, see also EHRENBERG *et al.*, 1949; GUSTAFSSON *et al.*, 1950.

Different methods of isotope application have been tried.

(1) In the middle of March, when the buds start their swelling, bare apple twigs (scions) of about 25 cm length were cut and placed in P32 solution in room temperature. The intention was to graft the scions into apple trees in the field as soon as a good uptake of P32 could be established. The material treated in this way would then constitute a good parallel to the X-ray and neutron scions. The uptake and transport rate proved, however, very slow. In a typical experiment (Table 1) 10 twigs were placed in 150 ml of solution containing 10×40 μC of P32. The concentration of P32, recalculated to the date when the experiment was started, was determined in buds of different position, *viz.*

TABLE 1. *P32 uptake of cut apple twigs from solutions or through a glass capillary.*

Amount of P32 given on March 12th	Position of buds analysed. *Cf.* text	Specific activity in μC/g (recalculated to the start of the experiment)		
		Buds cut on March		
		15th	18th	31st
40 μC/twig from solution	a	0	0	0,46
	b	0	0,008	0,56
	c	0	0,053	1,33
40 μC through glass tube	d	4,6	2,4 [1]	
	e	0,49	0,31 [1]	
	f	0,026	0,014 [1]	

[1] This decrease is due to a swelling of the buds; the absolute amount of P32 is greater than before, or unaltered.

a=two buds next to the top bud, *b*=two buds on the middle of the twig, and *c*=two buds next to the surface of the solution. The uptake was not appreciable until after about 3 weeks. At that date the twigs had started to wither and die and could no longer be used as scions for grafting.

Experiments performed later in the vegetation period have demonstrated that P as well as S uptake from solutions is appreciable only in leaf-carrying twigs. Such twigs cannot be used for grafting either. The possibility of budding remains, however, in this latter case, which can be of some interest but has not been utilized so far.

(2) In Table 1 are also given the results of an experiment, where the same amount of P32 as was given in the previous experiment was distributed through a glass tube inserted into the stems of pot trees. In Table 1 *d* denotes two buds just above the insertion point, *e* just below it, and *f* two buds 20 cm below the tip of the tube. This method is too laborious to be used on a large scale, but the results clearly show a much better uptake, and they also emphasize the fact that the main sap stream flows upwards.

(3) In another set of experiments, equally performed in the spring but on trees growing in the field, solutions of P32 were injected into leaf and flower buds, in the cushionlike bud-bases, or into the internodes of twigs. The injections were made with an »Agla» micrometer syringe (from Burroughs Wellcome & Co., London; *cf.* Biochem. J., 19: 1111, 1925), which permits the injection of solutions of 0,001 ml with an

accuracy of 5 per cent. Before the injection, a narrow perforation was made with a syringe needle or a drill. Although, in work with concentrated isotope solutions, a fairly large amount of the radiation source can be brought into meristematic regions, there are many disadvantages with the injections into buds and »cushions», *viz.* the very limited volume that can be injected, the hydrostatic pressure of the plant causing an exudation of already injected material — especially at moist weather the latter reason will make injections almost hopeless — and the mechanical injury due to the injection *per se*, causing the death of many buds already when pure water is injected into them (*cf.* p. 413 and Tables 7, 10, and 11).

Injections into buds of *Prunus Avium* on April 12th, 1949, of 2, 10, or 30 μC of P32 (in 0,0007, 0,0033, and 0,0100 ml, respectively) led to an absorption in the buds of 10—30 per cent of the P32 amount injected. In a few cases the fraction of P32 absorbed in the meristematic region of the bud was determined: 30—40 per cent of the P32 was found in this region, the weight of which is only 10 to 15 per cent of the weight of the bud, *i. e.* in this small region a P32 content of about 10 μC/g can be achieved.

Two typical experiments with injections in the bud cushions are summarized in Table 2. Only about one third of the amount given (10 μC per injection) is really taken up; the rest is exudated. Also, about one third of the P32 deposit is distributed to the bud, which thus contains about 1 μC. This amount is somewhat smaller in the case of apples, due to the fact that these trees have a later spring development. As the weight of buds at this time is about 50 mg for apples and 100 mg for pears, we get fairly high relative activities; data from other experiments give a mean value of 23 μC/g for apple buds and about 10μC/g for pear buds. In the latter experiments buds were cut for radioactivity

TABLE 2. *Distribution of P32 in twigs after injection of* < 10 μC *in each bud »cushion». Injection on 13th April, twigs cut for analysis on 19th April.*

Species	No. of injections	Average P32 content in μC (recalculated to injection date)			
		buds	cushions	internodes beneath bud	total
Moltke pears	10	1,08	1,04	1,19	3,31
Cox's Or. apples	6	0,78	1,91	0,98	3,67

analysis 8 and 19 days after injection. The measurements demonstrated that the transport of P32 from the injected deposit to the bud was nearly or totally finished after 8 days.

(4) Injections in branches and stems of 3—10 year old apple, pear and cherry trees were carried out in the field at the end of April and beginning of May when winter dormancy is relieved by sap movements and bud swelling. The active solutions of P32 and S35 were poured into bore-holes (diameter 3—8 mm) by the aid of the above-mentioned syringe. The hole was then immediately covered up with grafting-wax to prevent exudation. This method of introducing the isotopes is very easy to perform. Further, it causes negligible mechanical damage to the trees. Some losses of isotope material at the injection sites cannot be avoided, but the method is surely of practical value for the purpose in question, as the isotope concentration (of P32 as well as S35) in buds and other meristematic regions has proved much higher than the average value for a whole tree or branch (*cf.* p. 404). By the aid of a portable Geiger-Müller instrument (»Boreniimeter»; see the following section) it was also possible to get a good survey of the isotope uptake and distribution in different parts of the trees. In 8-year-old apple trees (trunk diameter 4,5—6,5 cm at the injection sites; 30—40 cm above ground; height 1,8—2,5 m; weight above injection sites 5—8 kg) which were injected with P32 on May 3rd, 1951, measurable quantities could be traced in the main branches 20—100 cm higher up 26—28 hours after injection. Next day, 50—54 hours after injection, the transport had reached points at a distance of 35—180 cm from the bore-holes. It was often observed that branches inserted near and straight above an injection site obtained a quicker and richer supply of the isotope than those inserted at the opposite side of the trunk. In many cases, however, a spiralization of the upward stream could be observed.

In order to test the validity of the general physiological rule that the upward stream is carried out by the vessels in the xylem, ringing of the bark was performed on two main branches of an apple tree (C 0206, Tetraploid 37/60) and 2000 μC of P32 were injected (a) immediately above the ring, (b) immediately below the ring, respectively. A control branch (c) without ring was also injected with the same isotope quantity. After 48 hours quite clear deflexions were read on the »Boreniimeter» at the tops of all three branches, thus indicating that the interruption of the phloem tubes played a negligible role at this first transport. Eight days later, however, traces of P32 could be found in twigs below the injection sites of main branches b and c, but not in similarly situated twigs

of main branch a. The explanation most obvious seems to be that the downward stream in the sieve tubes, starting after the development of the leaves for the transport of assimilates, also conveyed some radiophosphorus, which was then taken over by the vessels in lower branches.

In a greenhouse experiment in the early spring of 1952 the relative P32 and S35 contents of different parts of potted apple and cherry trees were followed for a couple of months after the injection of radioactive material. Originally eleven trees were selected for the experiment — seven apple and four cherry trees — but the very early forcing of the trees in January caused some irregularity and defectiveness of the awakening from winter dormancy in five of the apple trees. In the normally developing two apple and four cherry trees measurements were made on buds at different distances from the sites of injection, and the isotope content could be plotted as a function of time after injection; *cf.* Table 5. The data are expressed in mrh values, which are proportional to the isotope concentration; see the following section. It is seen that in those buds which were situated close to the injection sites, the radioisotope concentration rapidly rose to a very high value, whereas in the more distant buds the uptake was slower. The importance of making the injections at an appropriate state of development was also demonstrated. The apple trees, Nos. 3 and 9, were treated before the start of sprouting, whereas the cherry trees, Nos. 5 and 11, which showed the highest uptake rate, were treated after this stage. It can also be stated that the sulphur isotope is absorbed much more slowly than P32. This fact in addition to the long half-life of S35 leads to a very extended irradiation time.

The above-mentioned secondary downward isotope transport was evident also in the potted trees of this experiment as exemplified in Fig. 33 by the buds 1—5 of cherry tree No. 5, and in Fig. 34 by the buds 15, 17, and 18 of apple tree No. 9. The same is visualized in Table 3 c, buds 9: 11 and 12 and in Table 5, buds 5: 5 (= a) and 9: 18 (= b). The greatly preponderating isotope quantity does, however, stay in the tissues above the injection sites [*cf.* also p. 388, point (2)].

THE DETERMINATION OF β-PARTICLE DOSES.

For the determination in the field and in the glasshouses of the relative isotope content of different parts of the treated trees a portable Geiger-Müller apparatus was constructed by Mr. S. BORENIUS, Engineer, Lidingö; hence the instrument is called »Boreniimeter». The G.-M. tube

is connected through a flexible one-meter cable to a counting rate meter instrument. The counter tube GMT 82 (from The General Electric Co. Ltd.) was used; this tube is sufficiently thin-windowed to make measurements of the low energy β-radiation from S35 possible. As its window consists of aluminium, it is at the same time insensitive to strong daylight. The meter deflexions of the instrument were calibrated in mrh (milliroentgens per hour) by aid of a radium source. A conversion factor from mrh to μC per g of tissue was then determined empirically through analysis in the usual way of the content of the radioactive element in objects giving different scale deflexions when measured with the »Boreniimeter». Provided the counter tube of the latter is held alike in all measurements (we found it suitable to hold it as close to the object as possible), and provided the radiation from other parts of the tree is screened off (which can easily be performed with the hands), a sufficiently constant relation between the isotope concentration and the mrh value is obtained (Tables 3 a—c).

In this connection it may be pointed out that for a dose calculation the radioisotope content actual at the moment of measurement has to be determined. When the isotopes, on the other hand, are used as tracers, *e. g.* in a discussion of phosphate or sulphate transport, all measurements have to be recalculated to one and the same moment.

TABLE 3 a. (*P32.*)

Tree and bud	Date of sampling	Variety	Development stage of sample	mrh (Boreniimeter)	μC/g	μC/g and mrh
4 : 5	4.II	Cherry, Napoleon	Swelling leaf and flower buds	200	14,0	0,070
4 : 5	»	» »	Bud base, spur	25,5	2,64	0,104
4 : 10	»	» »	Swelling leaf and flower bud	1,41	0,089	0,063
4 : 8	11.II	» »	Breaking leaf and flower bud	64	5,93	0,093
4 : 18	22.II	» »	Flowers in blossom	5,6	0,80	0,143
4 : 19	»	» »	Developed leaves	16,4	1,59	0,097
4 : 19	»	» »	Fruitlets in petal fall	28,2	2,61	0,093
5 : 14	10.III	» »	Developed leaves	14,9	0,948	0,064
5 : 14	»	» »	Fruitlets	15,7	0,754	0,048
3 : 18	11.II	Apple, Tetr. $^{38}/_{1}$	Green flower cluster	80	7,62	0,095
3 : 29	22.II	» »	Pink flower buds	17,2	1,38	0,080
3 : 29	»	» »	Developed leaves	14,2	1,48	0,104
3 : 3	10.III	» »	» »	0,36	0,0216	0,060
3 : 3	»	» »	Small fruitlets	0,17	0,0167	0,098
3 : 27	»	» »	Flowers in blossom	2,4	0,253	0,105
			Mean of 15 determinations (P32):			0,0878

TABLE 3 b. (*S35.*)

Tree and bud	Date of sampling	Variety	Development stage of sample	mrh (Boreniimeter)	μC/g	μC/g and mrh
11 : 2	22.II	Cherry, Erianne	Developed leaves	2,4	4,84	2,02
11 : 3	»	» , »	Fruitlets in petal fall	3,0	18,78	6,26
11 : 3	»	» , »	Flowers in blossom	7,0	20,66	2,95
11 : 10	10.III	» , »	Developed leaves	2,4	6,51	2,71
10 : 12	22.III	» , Annonay	» »	2,1	3,58	1,70
10 : 24	»	» , »	» »	2,7	5,03	1,86
10 : 26	»	» , »	» »	3,3	5,48	1,66
10 : 30	»	» , »	» »	0,12	0,401	3,34
10 : 34	»	» , »	» »	0,77	1,85	2,40
11 : 4	»	» , Erianne	» »	4,1	7,14	1,74
11 : 10	»	» , »	» »	3,6	3,92	1,09
11 : 12	»	» , »	» »	6,6	11,43	1,73
11 : 20	»	» , »	» »	2,1	7,33	3,49
11 : 21	»	» , »	» »	5,6	15,22	2,72
7 : 4	22.II	Apple, Tetr. $^{37}/_{48}$	Leaf bud, green cluster	3,0	2,48	0,83
7 : 5	»	» , »	Flower bud, green cluster	0,59	0,883	1,50
9 : 14	10.III	» , Tetr. $^{38}/_{10}$	Developed leaves	1,07	1,21	1,13
9 : 20	»	» , »	» »	9,0	7,20	0,80
8 : 11	»	» , »	» »	1,2	2,07	1,73
7 : 2	22.III	» , Tetr. $^{37}/_{48}$	» »	0,65	1,60	2,46
7 : 11	»	» , »	Fruitlet	0,12	0,133	1,11
7 : 11	»	» , »	Developed leaves	0,12	0,392	3,27
7 : 12	»	» , »	» »	0,47	1,29	2,74
7 : 12	»	» , »	Fruitlet	0,59	0,714	1,21
7 : 13	»	» , »	Developed leaves	0,41	1,01	2,46
7 : 13	»	» , »	Fruitlet	0,17	0,266	1,56
9 : 19	»	» , Tetr. $^{38}/_{10}$	Developed leaves	8,0	15,86	1,98
9 : 21	»	» , »	» »	2,1	7,95	3,79
9 : 23	»	» , »	» »	3,6	10,00	2,78
9 : 26	»	» , »	» »	2,1	4,62	2,20
			Mean of 30 determinations (S35):			2,241

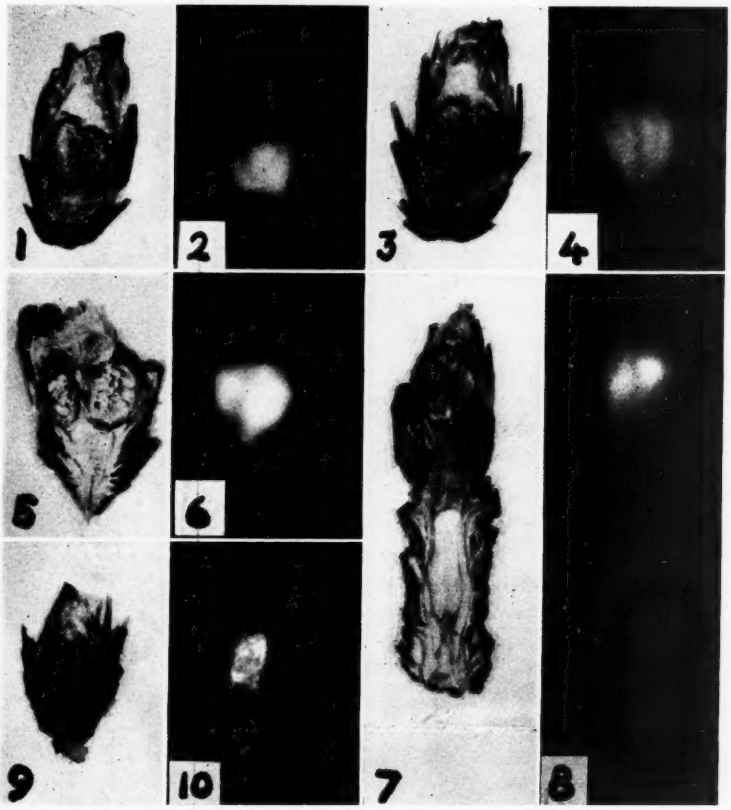
In the case of P32 (Table 3 a) the conversion factor of 0,088 μC per g per mrh is independent of the species (pot trees of apples and cherries investigated), and developmental stage of the object (buds, twigs, leaves, flowers, and fruitlets give about the same value). During the first months after injection of P32 into a tree the concentration of the isotope in any part of the tree at any time can be determined with an accuracy of about 30 per cent.

TABLE 3 c. (*S35.*)

Tree and bud	Date of sampling	Variety	Development stage of sample	mrh (Borenii-meter)	μC/g	μC/g and mrh
11 : 5	4.II	Cherry, Erianne	Breaking leaf and flower bud	0	5,9	—
11 : 5	»	» , »	Bud base, spur	0	3,6	—
11 : 6	»	» , »	Swelling leaf and flower bud	0	9,16	—
11 : 8	»	» , »	Swelling leaf and flower bud	0	3,93	—
10 : 31	11.II	» , Annonay	Leaf buds in dense cluster	0	3,94	—
11 : 6	»	» , Erianne	Bursting flower bud	0,72	17,85	24,8
11 : 16	»	» , »	» » »	0,89	9,56	10,7
11 : 18	»	» , »	» leaf buds	0,72	12,94	18,0
10 : 5	22.II	» , Annonay	Dormant bud	0	0,0551	—
11 : 15	»	» , Erianne	Swelling bud	0	0,636	—
9 : 9	4.II	Apple, Tetr. $^{38}/_{10}$	Dormant bud	0	0,0036	—
9 : 10	»	» , »	» »	0	0,00020	—
7 : 1	11.II	» , Tetr. $^{37}/_{48}$	Flower buds in dense cluster	0	0,0439	—
7 : 3	»	» , »	Breaking leaf bud	0	0,498	—
9:11[1]	10.III	» , Tetr. $^{38}/_{10}$	Small fruitlet	0	0,0228	—
9:12[1]	»	» , »	Developed leaves	0	0,0924	—
			Mean of 3 determinations (S35):			17,8

[1] Buds below injection sites; see Fig. 34.

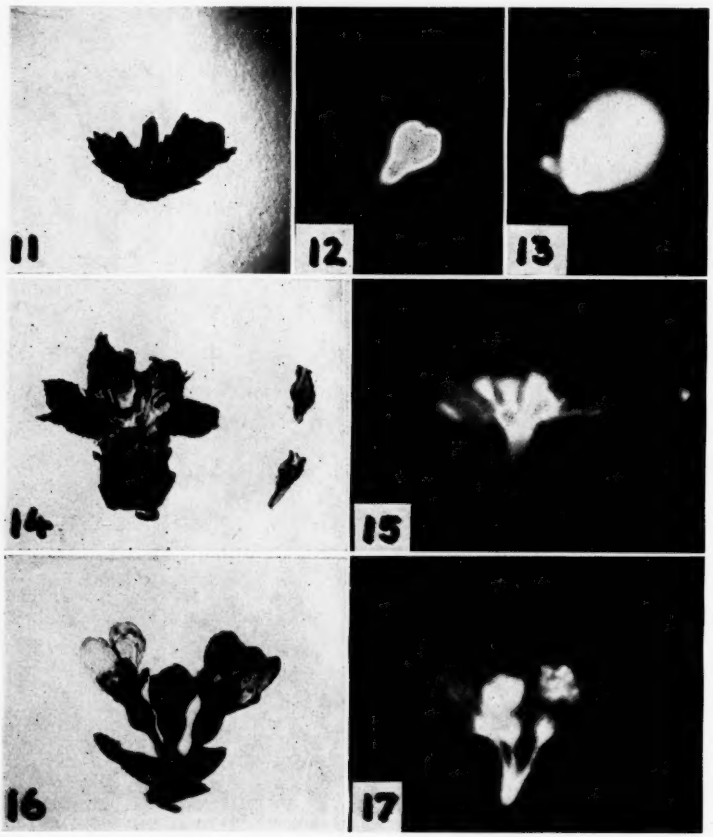
When the dose of the energetic β-particles from P32 is to be calculated, allowance has to be made for the fact that the organs which have absorbed the isotope, cannot be regarded large compared to the range of the radiation. (The straight path of mean energy particles is 2,7 mm, of maximum energy particles 7 mm; the effective ranges of the particles are about 40 per cent shorter.) It can be calculated (*cf.* SIRI, 1949, p. 435) that in a sphere of 1 mm radius containing uniformly distributed P32 the dose rate at the centre will be insignificantly (about 10 per cent) lower than at a point inside a large body with the same P32 concentration. At the surface of the sphere the dose rate will be about half as great. In P32 treatment of root tips, the volumes of which are about 0,7 mm^3, and plants of barley and wheat RUSSEL and MARTIN (1949) and SPINKS *et al.* (1948) have calculated with an absorption of about 50 per cent of the energy. In the present case the volumes (buds, *etc.*) containing the radioactive material are seldom smaller than a mm radius sphere (*cf.* p. 403 and Figs. 1—25). We may, therefore, calculate with a variation of the dose rate within the sensitive regions between 50 and 90 per cent, average 70 per cent, of the maximum dose rate (*i. e.*



Figs. 1—10. Photographs and autoradiographs of swelling buds 5 days after injection. — Figs. 1—2, 3—4, 5—6: buds from the cluster at 5 in tree No. 4 (=4 : 5), cherry, P32. — Figs. 7—8: bud and spur 3 : 9, apple, P32. — Figs. 9—10: bud 11 : 5, cherry, S35. The concentration of P and S in the meristem is prominent.

the dose rate at a point inside a comparatively large volume containing uniformly distributed radioisotope material). Owing to the accumulation of the isotopes to meristematical regions (*cf.* p. 402 ff.) the dose may be higher in some cases where most of the isotope material is concentrated to a small spot of a larger object. Owing to the long range of the P32 β-particles concentration differences between volume elements smaller than 1 mm^3 will be cancelled.

We state that the average P32 β-particle doses can be determined



Figs. 11—17. Photographs and autoradiographs of buds and flowers 13 days after injection of P32. — Figs. 11—13: bud 4 : 8, cherry. — Figs. 14—15: bud 3 : 26, apple. — Figs. 16—17: flowers from bud 4 : 17, cherry.

with an accuracy of about 30 per cent (standard deviation of the values in Table 3 a $=0{,}024$, corresponding to 27,3 per cent of the average $0{,}088$); within different parts of the organs irradiated the dose varies with about 30 per cent in both directions. This determination will be sufficiently reliable for semi-quantitative comparisons with other sorts of radiation.

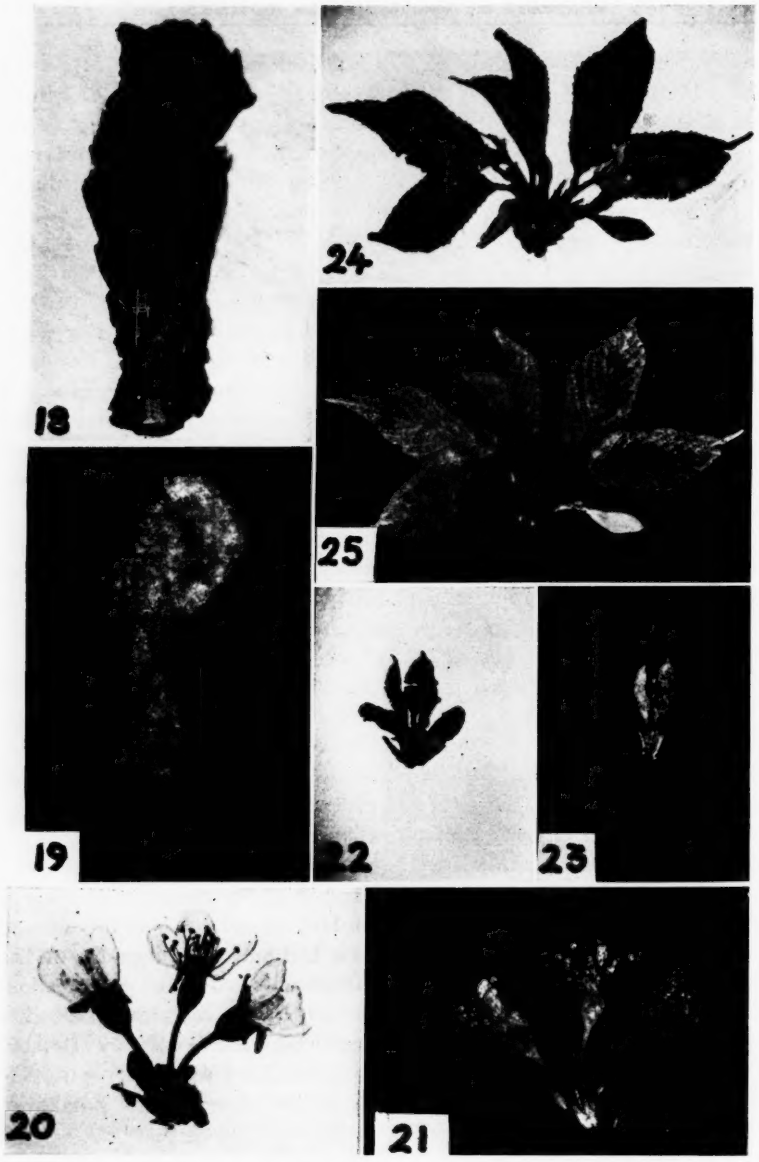
Owing to the very high absorption of the low energy S35 radiation the relation between μC/g of tissue/mrh is more dependent on the nature of the object (Table 3 b). The isotope content of unsprouted buds cannot

be measured at all, because all the S35 is localized in the meristem (*cf.* the following section; Figs. 9—10), and all energy is absorbed before reaching the surface of the bud (Table 3 c). A factor of 2 μC/g of tissue/mrh would be useful for the determination of the size of order of the dose. (The values about 18 μC/g-mrh in Table 3 c are determined at the moment of sprouting.) To gain a greater accuracy in the dose determination (standard deviation of the values in Table 3 b = 1,109, corresponding to 49,5 per cent of the average 2,24) corrections regarding the anatomical characteristics of the object should be made. On the other hand, the determination of the S35 radiation dose gains in accuracy through the fact that nearly 100 per cent of the energy is absorbed within the tissue. The short range of the S35 β-particles (mean range about 0,02 mm; *cf.* GUSTAFSSON *et al.*, 1950) would cause a great error in the dose determination in cases where the isotope is concentrated to small volume elements of the tissue. The sulphur isotope is, however, much more evenly distributed than the phosphorus.

In the dose calculations we have used the mean energy values of 0,7 MeV for the P32 and 0,033 MeV for the S35 β-rays (*cf.* EHRENBERG *et al.*, 1952). From the integrals of the curves, exemplified through the values in Table 5, the doses in roentgen equivalents given in Table 4 have been calculated. The rep unit (roentgen equivalent physical; *cf.* EVANS, 1948) has been regarded as the absorption of 93 ergs per g of tissue (National Bureau of Standards, Handbook 42, 1949). The shape of the *concentration-time* curves is a very complicated function of several factors, *viz.*: (a) isotope uptake; (b) isotope decay; (c) growth of the tissue, causing a dilution of the radioactive substance; (d) an emission of the material through the downward sap-stream has been found negligible (*cf.* above, p. 391) here; (e) in the case of dying buds special effects, as inhibition of growth and drying of the tissue, may cause a second rise of the curve (*cf.* Table 5 b, bud No. 11 : 3).

The dose integrals are determined through measurement of the area of the curve. To the value thus obtained is added, as a correction, the decay integral of the isotope in energy units, calculated from the last day of measurement; in this correction the contrarily directed factors (a) and (c) are neglected. In the case of the slowly decaying S35 this decay integral is not included in the dose values but given separately (Table 4).

It can be concluded that by a technique easily performed it is possible to give P32 as well as S35 β-particle doses equivalent to thousands r of X-rays, *i. e.* a range which is convenient in radio-biolog-



Figs. 18—25. Photographs and autoradiographs of buds, flowers and leaves 13 days after injection of S35. — Figs. 18—19: breaking bud with spur 7 : 2, apple. — Figs. 20—21; flowers of bud 10 : 7, cherry. — Figs. 22—23: leaves in development from bud 11 : 18, cherry. — Figs. 24—25: more developed leaves from 10 : 31, cherry.

TABLE 4. *Integral doses, in rep, in different buds. The cross (†) means that the bud has died during the period of investigation. In the case of the slowly decaying S35 the decay integral based on the last μC/g determination is added in brackets. In the case of P32 this integral is insignificant and included in the dose value.*

Species; tree No. (*cf.* Figs. 31—36)	Isotope	Amount injected, μC	No. of bore-holes	Weight of tree or branch g	Radiation dose calculated, rep							
					Average for tree or branch	In buds, enumerated from base to top						
						a	b	c	d	e	f	g
Apple 3A	P32	500	3	70	4000	6600	3700	870	660	2200	—	—
» 3B	»	500	3	70	4000	2700	1900	1300	840	930	—	—
» 3C	»	500	3	70	4000	7700	1600	1600	650	660	—	—
Cherry 4	»	1500	1	100	8300	34000†	170†	5700	3700	6100†	15000	2000
» 5	»	1500	4	120	6900	450†	18000†	10000†	6800†	8300	6600	4900
Apple 9	S35	6000	5	100	13500	570	1050	2200	2900	1100	800	560
						(+ 460)	(+ 760)	(+ 2300)	(+ 3300)	(+ 560)	(+ 1100)	(+ 510)
Cherry 10	»	740	1	160	1000	280	1200	360	750	520	330	—
						(+ 100)	(+ 560)	(+ 150)	(+ 400)	(+ 150)	(+ 120)	
» 11	»	1800	4	100	4000	800†	1000	1200	2700	1700	1400	1500
							(+ 460)	(+ 490)	(+ 1500)	(+ 760)	(+ 510)	(+ 970)

TABLES 5 a and b. *Examples of radioactivity measurements on one apple branch (3A; cf. Fig. 31) and one cherry tree (5; cf. Fig. 33), treated with P32, as well as one apple (9; cf. Fig. 34) and one cherry tree (11; cf. Fig. 36), treated with S35. In brackets are given mrh values which should have been obtained in case of a homogeneous distribution of the isotope. The dose integrals are given in Table 4.*

5 a: P32 treatment.

Tree and bud No. (letters a–g refer to Table 4 and Figs. 31, 33, 34, 36)	mrh values, at days after injection									Remarks
	3	5	8	11	17	20	29	51	82	
3 A:	(70)	(64)	(55)	(48)	(36)	(31)	(20)	(6,9)	—	—
Apple 5 (a)	0,9	1,5	48	72	100	112	48	13,4	—	—
6 (b)	0,3	2,2	12,0	80	64	72	23	2,8	—	—
7 (c)	0	0	0,5	0,5	3,9	8,0	10,5	4,3	—	—
10 (d)	0	0	0	0	0	0,3	12,7	2,6	—	Shoot 27 cm after 117 days
17 (e)	0	4,1	15,2	18,3	23	21	13,7	1,6	—	—
5:	(123)	(112)	(97)	(83)	(62)	—	(36)	(12,0)	(2,8)	—
Cherry 5 (a)	0	0	1,1	2,5	5,0	—	3,2	3,0	0,1*†	Bud below injection sites *Ripe fruits. † Dead after 85 days
6 (b)	15,8	90	220	360	275	—	170	36†	4,5†	† Dead after 45 days
8 (c)	6,3	64	80	200	220	—	80	13,4†	0,7†	† Dead after 50 days
12 (d)	0	5,0	30	56	100	—	80	8,4	0,5	† Dead after 85 days
14 (e)	0	1,2	41	125	112	—	90	7,0	2,0*	*Ripe fruits. — Shoot 2 cm after 117 days
9:	(70)	(64)	(55)	(48)	(36)	(31)	(20)	(6,9)	—	—
Apple 1 (a)	0,3	1,2	12,0	80	64	72	23	2,8	—	—
2 (b)	0,9	1,5	48	72	100	112	48	13,4	—	—
3 (c)	0	0	0,5	0,5	3,9	8,0	10,5	4,3	—	—
4 (d)	0	0	0	0	0	0,3	12,7	2,6	—	Shoot 27 cm after 117 days
5 (e)	0	4,1	15,2	18,3	23	21	13,7	1,6	—	—
11:	(123)	(112)	(97)	(83)	(62)	—	(36)	(12,0)	(2,8)	—
Cherry 1 (a)	0	0	1,1	2,5	5,0	—	3,2	3,0	0,1*†	* Ripe fruits † Dead after 85 days
2 (b)	15,8	90	220	360	275	—	170	36†	4,5†	† Dead after 45 days
3 (c)	6,3	64	80	200	220	—	80	13,4†	0,7†	† Dead after 50 days
4 (d)	0	5,0	30	56	100	—	80	8,4	0,5	† Dead after 85 days
5 (e)	0	1,2	41	125	112	—	90	7,0	2,0*	*Ripe fruits. — Shoot 2 cm after 117 days

12 (d)	0	5,0	30	56	100	—	80	8,4	0,5	† Dead after 85 days
14 (e)	0	1,2	41	125	112	—	90	7,0	2,0*	*Ripe fruits. — Shoot 2 cm after 117 days
17 (f)	0	1,2	30	80	112	—	56	12,6	1,1*	* Ripe fruits
18 (g)	0	0	14,2	41	41	—	72	4,8	3,3	Shoot 22 cm after 117 days

5 b: S35 treatment.

	17	20	29	33	51	54	62	82	117	
9:	(24)	—	—	(21)	(18)	—	(16)	—	(11)	—
Apple 18 (a)	0	—	—	0,9	1,2	—	2,7	—	0,9	Bud below injection sites
1 (b)	1,1	—	—	7,0	2,1	—	1,8	—	1,5	Shoot 3 cm after 117 days
28 (c)	0	—	—	11,2	4,2	—	7,0	—	4,6	—
19 (d)	0	—	—	11,2	6,3	—	9,8	—	6,4	—
21 (e)	0	—	—	5,0	2,7	—	4,6	—	1,1	Shoot 2 cm after 117 days
22 (f)	0	—	—	1,2	1,8	—	2,9	—	2,2	Shoot 2 cm after 117 days
26 (g)	0	—	—	3,6	1,3	—	1,6	—	1,0	Shoot 4 cm after 117 days
11:	(7,2)	(6,9)	(6,4)	—	(5,4)	(5,2)	(4,9)	(4,2)	(3,2)	—
Cherry 3 (a)	4,2	6,3	2,7	—	9,0†	—	—	—	—	† Dead after 45 days
4 (b)	1,2	3,0	3,3	—	3,0	2,6	2,4	2,7	0,9	—
10 (c)	1,9	3,4	1,3	—	2,8	2,9	3,5	3,6	1,2	Shoot 2 cm after 117 days
12 (d)	1,9	3,6	5,3	—	7,6	8,3	7,8	9,0	3,0	—
17 (e)	2,2	3,6	2,7	—	3,9	4,8	4,5	6,3	1,5	—
19 (f)	3,0	4,2	3,4	—	3,6	3,5	3,9	4,1	1,0	—
21 (g)	1,9	4,3	3,0	—	3,0	4,1	4,8	5,0	1,9	Shoot 2 cm after 117 days

ical work. Through a simple technique the doses can be measured with an accuracy sufficient for semi-quantitative or — after the performance of certain corrections — quantitative comparisons with other radiations. From the isolated analyses and measurements performed it can further be concluded that in the earlier material discussed in the previous chapter and in connection with the biological effects (p. 409), many buds have received doses of the magnitude given in Table 4, *i. e.* in the case of P32 500—5000 rep and in the case of S35 200—2000 rep.

THE DISTRIBUTION OF P32 AND S35.

Different measurements, already mentioned, have clearly demonstrated that there is an accumulation of P32 and S35 when given as

TABLE 6. *Particulars concerning the autoradiographs. Injections on 30. I. 1952 on pot trees.*

Fig. No.	Species	Description of specimen	Specimen taken	Isotope	Average content of isotope μC/g	Exposition hrs.	Development mins.	Magnification of the fig.
2	Cherry	Early flower bud	2.II	P32	14	1,5	1	3
4	»	»	»	»	14	1,5	1	3
6	»	» (Fixed in ethanol)	»	»	14	1,5	1	3
8	Apple	Early flower bud with spur	»	»	0,35 0,04	1,5	5	3
10	Cherry	Early flower bud	»	S35	5,9	3	—	3
12—13	»	Group of three leaf buds	11.II	P32	6,7	1,7 17	1	1
15	Apple	Group of flower buds, two of which separate	»	»	0,52	2	5	1
17	Cherry	Developing flowers; left flower cut longitudinally	»	»	30,2	1,7	1	1
19	Apple	Sprouting leaf bud	»	S35	0,2 [1]	17	5	4
21	Cherry	Flowers; middle flower opened	»	»	—	17	1	1
23	»	Leaf bud	13.II	»	13	17	0,5	1
25	»	Leaves	11.II	»	3,8	17	0,75	1

[1] Only adjacent buds analysed.

phosphate and sulphate, respectively, to meristematic regions of the trees. As this fact is of great importance for the practical use of the isotopes with genetical purposes, some data have to be given here (*cf.* the work on P32 and S35 distribution in barley by EHRENBERG *et al.*, 1949, and GUSTAFSSON *et al.*, 1950).

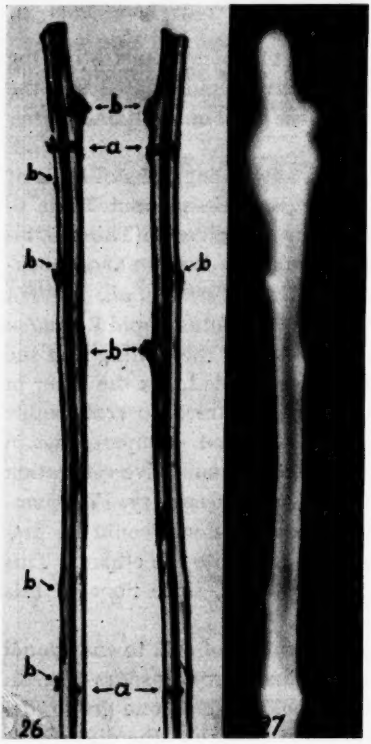
(1) Direct analyses of the P32 content of cherry buds after the injection of the isotope into the »cushions» have demonstrated that the concentration of P32 in the embryonal tissue is about 5 times higher than in the rest of the bud (*cf.* p. 389).

(2) The same effect is demonstrated in autoradiographs of buds of different origin and state of development (Figs. 1—27 and Table 6, where particulars of material and technique are given). (These autoradiographs were made by Amanuensis MARGARETA BRUNNBERG, Stockholm.) They are prepared as described by GUSTAFSSON *et al.*, 1950; it suffices to add that for smaller objects Gevaert's Dentus Rapid Roentgen film was used. As 1—2 days always pass between the cutting and the autoradiographing of the buds, an attempt was made to fix the latter in abs. ethanol at the moment of cutting from the tree. No remarkable differences from unfixed material could be observed (compare Figs. 6 — fixed — with 2 and 4 — unfixed), so that for a qualitative estimation of the isotope distribution fixation does not seem necessary. For quantitative measurements of P distribution freeze sections would be preferable as 5—10 per cent of the P32 were eluated into the ethanol. This fixative can be used in the case of S35, however, since none of this isotope is eluated.

Figs. 1—8, 11—13 visualize the concentrating of P32 to embryonal parts of buds, and not to, *e. g.*, bud scales or bud-carrying spurs (Fig. 8). The very differential P32 uptake of buds belonging to one group, demonstrated in Figs. 11—13, is extraordinary; as a rule, the differences between buds of one group but which develop at very different rates are not so great. In flower buds (Figs. 14—15) and developing flowers (Figs. 16—17) the P32 concentration is highest in ovaries, flower stalks (especially the bases of the latter), anthers, and filaments. The distribution of S35 is essentially similar. In the just sprouting leaf bud of Figs. 18—19 it is concentrated on the leaves, not on the scales; *cf.* the later developmental stages in Figs. 22—23 and 24—25. The high radiosulphur concentration in the bases of flower stalks of different development (Figs. 9—10 and 20—21) and also in the anthers (Fig. 21) is obvious.

(3) Autoradiographs of longitudinal sections of twigs (Figs. 26—27

and 38—39) demonstrate both for phosphate as well as for sulphate a certain lagging of the radioactive material at the injection sites (points *a* of Fig. 26), but also, besides the paths of spreading, an accumulation of the isotopes to the buds (points *b* of Fig. 26).



Figs. 26—27. Photographs and autoradiographs of longitudinal section of cherry twig about one week after P32 injection in 1950 (field tree). The activity is distributed all over the living tissues but especially concentrated on the injection sites (a) and buds (b).

The cherry twig pictured in Figs. 26—27 was cut about one week after the injections were made, and fixed in abs. ethanol before autoradiographing; only negligible amounts of P32 were eluated into the ethanol.

(4) The principle of an accumulation of the radioactive substances in question to meristematical zones is obvious from the fact already mentioned (p. 390) that the isotope concentration in buds and other growing parts of the tree is higher than the average concentration in the whole tree, calculated from a section just below the lowest injection site. This is valid in spite of the fact that a great part of the isotope material injected lags or is otherwise lost at the injection site [*cf.* point (3) above]. In Table 5, as well as in Figs. 28—36, the mrh values are calculated which would have been obtained in case of a uniform distribution of the isotope material within the tree or branch. Especially in the case of phosphorus treatment of cherries (trees 4 and 5; *cf.* Table 5 and Figs. 32—33), which was performed just after sprouting had started, a very high P32 concentration — and, consequently, dose (Table 4) — is found in the buds, compared to the average value. Although the difference is not so enormous, this is also evident in the case of the apple trees, which were treated at a somewhat earlier state of development (*cf.* the slower uptake rate!).

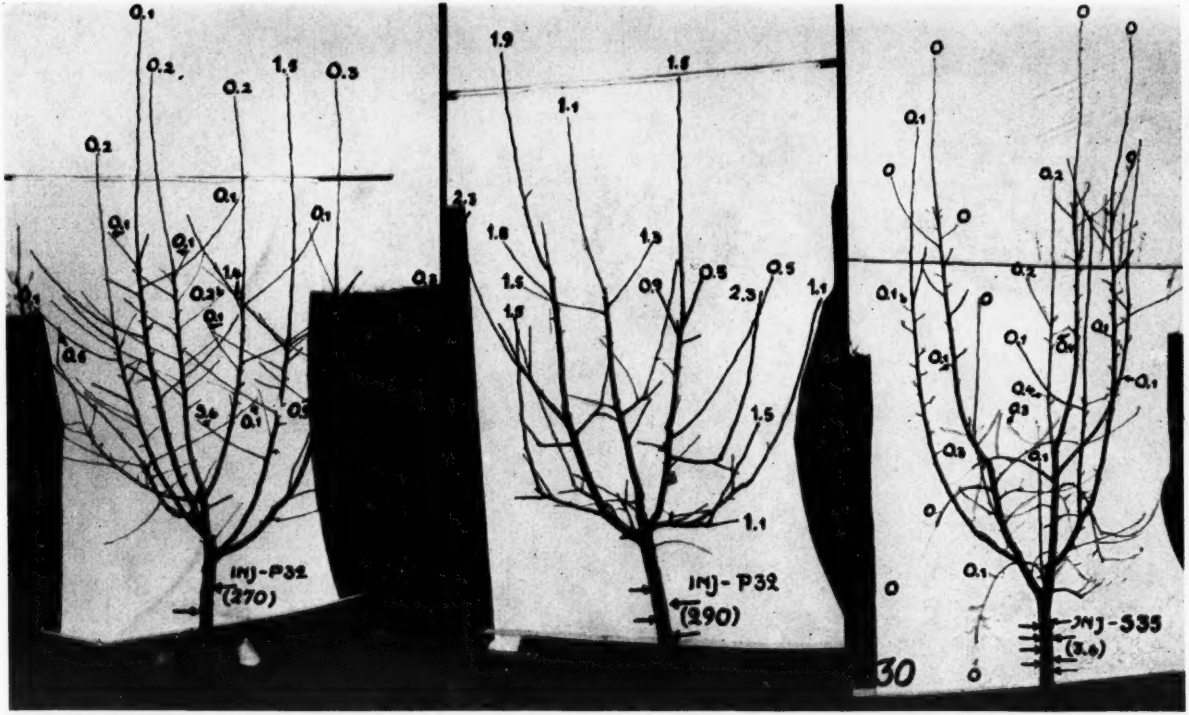


Fig. 28. The distribution of P32 in mrh 63 days after injection of the field tree C 1615 in 1951. Injection 4000 μC, 2 bore-holes; at uniform distribution, 0,3 mrh per bud. Photograph taken at the day of injection, May 3rd. Upper edge of the white screen 2 m above ground. Variety »Ingrid Marie» apple. — Fig. 29. The distribution of P32 in mrh 63 days after injection of the field tree C 1512. Injection 8000 μC, 4 bore-holes; at uniform distribution 0,9 mrh per bud. Variety »Ingrid Marie» apple. — Fig. 30. The distribution of S35 in mrh 63 days after injection of the field tree C 0417. Injection 12000 μC, 8 bore-holes. Variety »Ontario» apple.

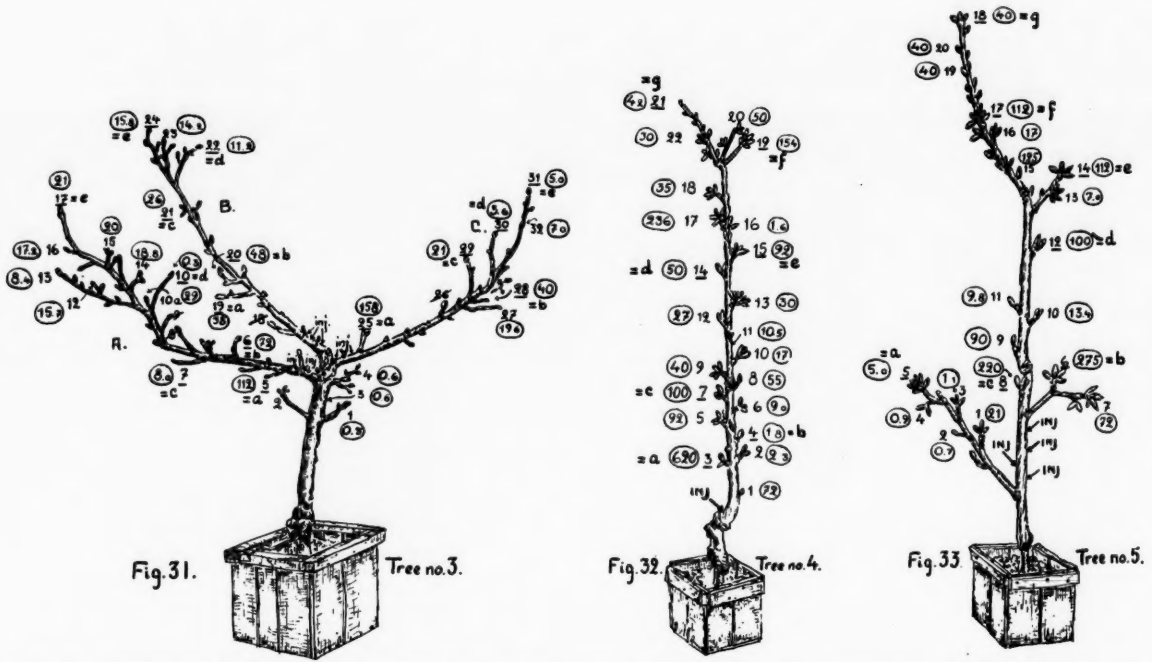
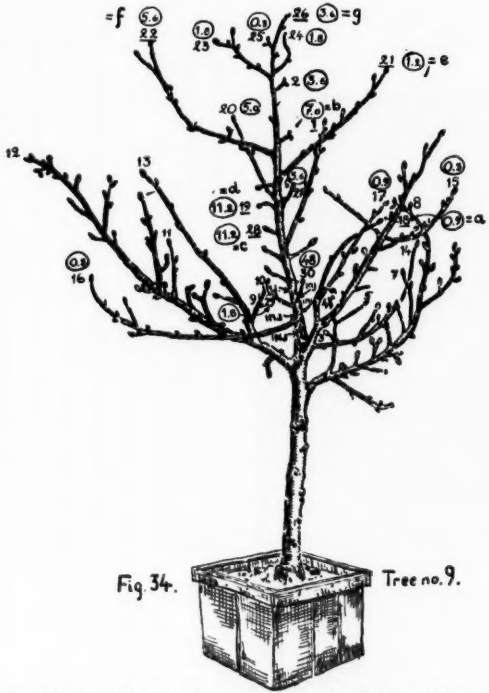
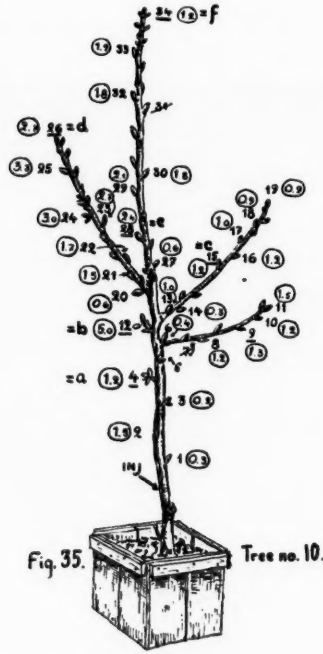


Fig. 31. The distribution of P32 (mrh values inscribed in circles) in the buds of the potted tree No. 3, 20 days after injection of 500 μC in each one of the main branches A—C; at uniform distribution 31 mrh per bud. Variety Tetraploid apple 38/1, figure drawn at injection, Jan. 30th, 1952. — Fig. 32. The distribution of P32 in the buds of the potted tree No. 4, 17 days after injection of 1500 μC in 1 bore-hole; at uniform distribution 75 mrh per bud. Variety »Napoleon» sweet cherry. — Fig. 33. The distribution of P32 in the buds of the potted tree No. 5, 17 days after injection of 1500 μC in 4 bore-holes; at uniform distribution 62 mrh per bud; *cf.* Table 5. Variety »Napoleon» sweet cherry. — (Drawings by K. ANJOU.)





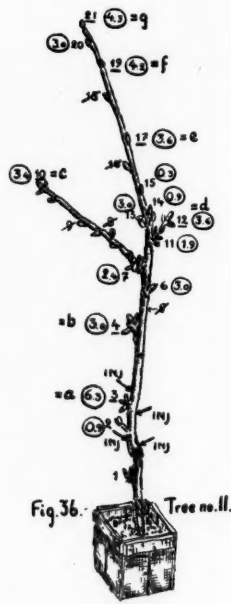


Fig. 34. The distribution of S35 in the buds of the potted tree No. 9, 33 days after injection of 6000 μC in 5 bore-holes of the top branch; at uniform distribution 21 mrh per bud; *cf.* Table 5. Variety Tetraploid apple 38/10. — Fig. 35. The distribution of S35 in the buds of the potted tree No. 10, 20 days after injection of 740 μC in 1 bore-hole; at uniform distribution 1,8 mrh per bud. Variety »Annonay» sweet cherry. — Fig. 36. The distribution of S35 in the buds of the potted tree No. 11, 20 days after injection of 1800 μC in 4 bore-holes; at uniform distribution 6,9 mrh per bud; *cf.* Table 5. Variety »Erianne» sweet cherry. — (Drawings by K. ANJOU.)

The sulphur isotope is more evenly distributed (*cf.* p. 397), although a concentration on the buds is observed, especially in the case of cherries

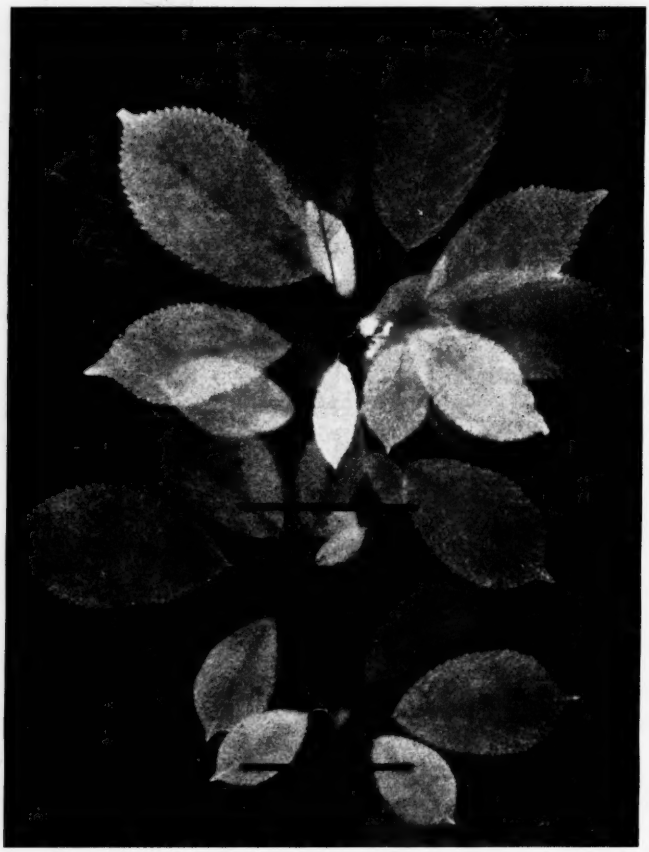


Fig. 37. Autoradiograph of cherry twig from the S35 injected potted tree No. 10. The portion between the black lines (including buds Nos. 10 : 8 and 10 : 9; *cf.* Fig. 35) is seen in magnification in Figs. 38—39.

(trees 10 and 11; *cf.* Figs. 35—36, also 37, 38—39). Similar data are obtained in trees treated in the field (Figs. 28—30).

As seen from the previous chapter, the uptake and distribution of a certain radioisotope dose are depending upon the method of supply

and the distances from the sites of supply. When the bore-hole injection technique was used distributions could be mapped as visualized by Figs. 28—30 (field trees) and 31—36 (potted trees). Tree C 1512 (Fig. 29) may serve as an example of a comparatively even distribution of P32 to the main branches. On the other hand, tree C 1615 (Fig. 28) shows that one of the main branches (emanating from a stem point straight above one of the injection holes) has received a much higher dosage of the same isotope (mrh values of the buds $3{,}_6$, $1{,}_4$, $1{,}_5$) than the other main branches (0—$0{,}_9$ mrh). In Figs. 32—33, where the mrh values of the day with maximal deflexions at the »Boreniimeter» are given for the different buds, the more even distribution of P32 in the tree with four bore-holes (Fig. 33) than in the one with the whole dose given through only one hole (Fig. 32) is obvious.

Figs. 38—39. Photograph and autoradiograph of longitudinal section of cherry twig from tree No. 10 (magnif. 3; *cf.* Fig. 37). The isotope S35 is accumulated in cambium and buds.

PRIMARY BIOLOGICAL EFFECTS OF DIFFERENT RADIATIONS.

Some facts concerning lethality and deformation effects in X-ray treated scions of apples, pears and cherries have already been reported in the papers by GRANHALL *et al.* (1949) and GRANHALL (1949, 1951). In the last-mentioned publications the first results from neutron treatment are also briefly described. In order to make comparison possible the isotope effects are now presented in Tables 7—11 together with an up-to-date survey of the corresponding phenomena in the X-ray and neutron treated material.

The injection of P32 in buds and cushions of apples (Table 7) was

TABLE 7. *Lethality and bifurcations induced in apples.*

Treatment and material	Dosage	Number of treatments	Surviving		Total No. of bifurc.	Treatments with bifurc.	
			No.	%		No.	per cent of surviv.
1. *X-rays*, scions 1947—51	0	46	37	80,5	0	0	0
	5000r	210	144	68,6	66	45	31,3
	7500r	223	69	30,9	9	8	13,0
2. *Neutrons*, scions 1949—51	0	49	40	81,6	0	0	0
	415—2020dis	225	200	88,9	4	4	2,0
	3000—5000dis	179	154	86,0	27	23	14,9
3. *Radiophosphorus*	0	22	17	77,3	0	0	0
a. buds, a. cushions 1949	2μC	75	54	72,2	2	2	3,7
	10μC	210	141	67,1	6	5	3,5
	30μC	7	1	14,3	1	1	100
b. branches 1950—51	0	5	5	100	1	1	20,0
	250—750μC	17	17	100	1	1	5,9
	1000—1600μC	13	13	100	0	0	0
	2000—3500μC	10	10	100	2	2	20,0
c. stems 1949, 1951	0	2	2	100	0	0	0
	1200—2400μC	7	7	100	9	4	57,1
	4000—8000μC	5	5	100	1	1	20,0
4. *Radiosulphur*	0	1	1	100	0	0	0
a. branches 1950—51	375—1000μC	7	7	100	0	0	0
	1500—2000μC	7	7	100	1	1	14,3
	3000—7200μC	8	7	87,5	0	0	0
b. stems 1951	3000—12000μC	3	3	100	0	0	0
5. *Untreated scions* 1950—51	—	555	548	98,7	4	3	0,5

TABLE 8. *Lethality and bifurcations in apples of different ploidy stages.*

Treatment and dosage	Survival percentage			Scions or buds with bifurcated shoots, per cent of surviving		
	Dipl.	Tripl.	Tetrapl.	Dipl.	Tripl.	Tetrapl.
X-rays; 5000—7500r	36,9	60,6	52,3	60,3	19,5	3,8
Neutrons; 3000—5000 dis	66,7	96,7	94,9	27,5	17,2	3,6
P32; 2—10μC per bud	54,1	77,3	85,3	4,1	5,2	1,6

TABLE 9. *Frequency of divided leaves in apples.*

Treatment and material	Dosage	Treated 1949				Treated 1950			Treated 1951	
		No. of surviv.	Treatment with div. leaves			No. of surviv.	Treatment with div. leaves		No. of surviv.	Treatment with div. leaves
			1949	1950	1951		1950	1951		1951
1. *X-rays*, scions	0	18	0	0	0	6	0	0	13	0
1949—51	5000r	59	1	0	0	—	—	—	—	—
	7500r	37	1	1	0	1	0	0	30	0
2. *Neutrons*, scions	0	13	0	0	0	13	0	0	13	0
1949—51	415—2020dis	118	7	2	1	83	1	0	—	—
	3000—5000dis	—	—	—	—	41	2	0	111	8
3. *Radiophosphorus*	0	14	0	0	0	—	—	—	—	—
a. buds a. cushions	2μC	35	1	0	0	—	—	—	—	—
1949	10μC	61	0	0	0	—	—	—	—	—
	30μC	1	0	0	0	—	—	—	—	—
b. branches	0	—	—	—	—	4	0	0	—	—
1950—51	250—750μC	—	—	—	—	12	2	2	6	0
	1000—1600μC	—	—	—	—	6	0	1	7	2
	1900—3500μC	—	—	—	—	5	0	3	8	2
c. stems	0	2	0	0	0	—	—	—	1	0
1949, 1951	1200—2400μC	4	0	4	1	—	—	—	3	1
	4000—8000μC	—	—	—	—	—	—	—	5	1
4. *Radiosulphur*	0	—	—	—	—	1	0	0	—	—
a. branches	375—1000μC	—	—	—	—	4	0	0	3	0
1950—51	1500—2000μC	—	—	—	—	4	0	1	3	0
	3000—7200μC	—	—	—	—	5	1	0	3	0
b. stems	3000—12000μC	—	—	—	—	—	—	—	3	0
1951										

TABLE 10. *Bifurcated shoots induced in pears.*

Treatment and material	Dosage	Number of treatments	Surviving		Total No. of bifurc.	Treatments with bifurc.	
			No.	%		No.	per cent of surviv.
1. *X-rays*, scions	0	45	28	62,2	9	0	0
1948—51	5000r	354	282	79,7	53	45	16,0
2. *Neutrons*, scions	0	35	32	91,4	0	0	0
1949—51	415—2020dis	150	135	90,0	5	5	3,7
	3000—5000dis	75	68	90,7	22	18	26,5
3. *Radiophosphorus*,	0	7	3	42,9	0	0	0
buds	2μC	55	26	47,3	1	1	3,8
1949	10μC	98	15	15,3	1	1	6,7

TABLE 11. *Bifurcated shoots induced in cherries.*

Treatment and material	Dosage	Number of treatments	Surviving		Total No. of bifurc.	Treatments with bifurc.	
			No.	%		No.	per cent of surviv.
1. *X-rays*, scions	0	51	24	47,1	0	0	0
1948—51	2500—3000r	179	136	76,0	15	13	9,6
	4000—5000r	112	35	31,3	6	5	13,3
2. *Neutrons*, scions	0	33	15	45,5	0	0	0
1949—50	310—1470dis	92	54	58,7	0	0	0
	1930—3100dis	104	58	55,8	1	1	1,7
3. *Radiophosphorus*	0	13	13	100	0	0	0
a. buds and cushions	2μC	75	69	92,0	0	0	0
1949	10μC	91	51	56,0	0	0	0
	30μC	16	12	75,0	0	0	0
b. branches	0	2	2	100	0	0	0
1951	250—1000μC	6	6	100	0	0	0
c. stems	0	2	2	100	0	0	0
1951	1000—2000μC	4	4	100	0	0	0
4. *Radiosulphur*	0	2	2	100	0	0	0
a. branches	375—1500μC	6	4	66,7	0	0	0
1950							
b. stems	600—6000μC	3	3	100	0	0	0
1950—51							

followed by a comparatively high lethality. The injection procedure in itself seems to a high degree responsible, as seen from the damage on the control buds which were treated with distilled water (77,3 per cent surviving). Similar results were also obtained with pears (Table 10). In cherries (Table 11), however, the 0 and 2 μC doses gave full or nearly full survival, which can be explained by the fact that the development was earlier and the buds already big and swollen. In apples a comparison of diploid, triploid and tetraploid varieties showed an increasing percentage of surviving P32 buds with higher ploidy (Table 8).

Isotope injection in bore-holes of branches and stems of apple and cherry trees (Tables 7 and 11) gave, at least in the higher concentrations, a very typical delay in the development, but only in three cases (branches into which large amounts of S35 were injected) lethal damage was induced. In some instances the β-particle doses have been high enough to kill individual buds (*cf.* Table 4). In the cases where buds having received low radiation doses also succumb, they are, as a rule, situated close to the injection site (buds 5 a, 11 a) or to a strongly irradiated bud (bud 4 b close to 4 a), *i.e.* their death is probably caused by damage in the underlying, nourishing tissue. Owing to the extremely variable growing conditions of individual buds their possibilities of surviving irradiation are very different (*cf.* discussion below, p. 416).

In the X-ray and neutron material the untreated scions were exposed to the same general temperature and humidity conditions during the time between cutting and grafting as were the irradiated scions. The surviving percentage was on an average about 80 in apples and pears and somewhat below 50 in the more sensitive cherries. As seen from the last line of Table 7, untreated apple scions which were grafted in a more ordinary and optimal way without desiccation showed a survival of 98,7 per cent. X-rays have a very keen effect upon the scion vitality. In apples, 5000 r lower the survival percentage to about 70, and 7500 r to about 30 (Table 7). If we consider the diploid varieties separately the corresponding values are about 50 and 20 per cent. The pears were only treated with 5000 r but seemed to endure this dose better than did the apples (Table 10). In cherries, finally, 4000—5000 r only left about 30 per cent of the scions alive, while 2500—3000 r had a remarkably weak lethal effect.

In contrast to the X-ray scions and the P32 bud injections but in resemblance to the isotope branch and stem injections the neutron-treated scions seemed just as viable as were the untreated controls. Although the neutron doses applied have given numerous disturbances

(see below), they have not been large enough to cause lethality. In spite of the comparative weakness of the neutron doses (5000 dis corresponds to about 200 rep; see discussion below), they suffice to demonstrate a reaction similar to that obtained in dormant barley seeds (GUSTAFSSON and MAC KEY, 1948; MAC KEY, 1951; EHRENBERG *et al.*, 1952), where severe damage (chromosome aberrations, sterility, genetical changes) are produced at doses which do not affect the survival. In this respect the different fruit species behaved quite alike at the applied doses.

At the growth of normal scions the new shoots very rarely form bifurcations, *i. e.* a splitting of the vegetation point in the top, thus giving rise to two (or more) terminal shoots, jointed at very pointed angles and, as a rule, conformably developing (pictures, see GRANHALL *et al.*, 1949). In the untreated ordinary apple material only 3 scions out of 548 survivors had such bifurcations (0,55 per cent). In all the radiation experiments the frequency was similar among the untreated controls of the different series (1 of 223 in total=0,45 per cent).

Among X-ray treated scions, however, bifurcated shoots were found in masses, as described by GRANHALL *et al.* (1949). The average frequencies, as compiled from Tables 7, 10, and 11, are for apples 24,9, pears 16,0 and cherries 10,5 per cent of treated scions. In apples it is quite evident that the higher ploidy stages are less liable to these developmental disturbances than are the diploids (Table 8).

The neutron treatment has caused a similarly increased formation of bifurcated shoots. In those scions which have been exposed to 3000 dis or higher doses the bifurcation frequencies are 14,9 in apples and 26,5 in pears. The other apple and pear scions and practically all cherry scions apparently received too low doses to show this effect. The relative sensitivity of the diploid apples is elucidated by Table 8.

Bifurcated shoots have also appeared in the isotope material but in a more capricious way, obviously depending upon the uneven distribution of the agent (*cf.* Tables 7, 10, and 11).

Most shoot bifurcations have appeared in the first vegetation season directly following upon grafting or injection. Several times the bifurcation was then repeated during the same or next season, and in a few cases the phenomenon appeared during three successive years. On the other hand, second year or later bifurcations are also noted. Especially remarkable was the sudden emergence in 1951 of forked shoots in two otherwise quite normal Boiken apple trees (C 0712 with two single, and C 0716 with one fourfold, bifurcations; both trees emanating from scions exposed to only 2500 r of X-rays in 1946).

It is interesting to note that FREISLEBEN and LEIN (1943) found bifurcated spikes in X-rayed barley and that the same phenomenon turned up in MAC KEY's (1951) barley material after X-ray as well as neutron treatment.

Leaf deformations have also been recorded in fruit trees in a great many cases; different types are pictured by GRANHALL *et al.* (1949). It is, however, often very difficult to distinguish the real radiation effects from the extremes of normal leaf-shape variation, especially in the first year development of graftings. In the years 1949—51 systematical annotations of the leaf abnormalities in the radiated material were performed, and in Table 9 an extract thereof is compiled, comprising only cases with forked midrib and divided lamina in apples. As seen there, most cases are first year effects, but many times divided leaves were also discovered the second or third year (or even later).

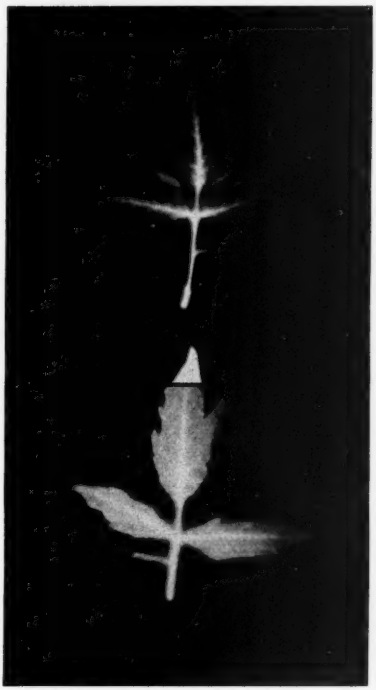


Fig. 40. Autoradiographs of one normal rose leaf from a plant treated with 300 μC of P32 (upper picture) and one abnormal leaf from another plant treated with 2000 μC. Because of weaker radiation the upper half of the picture was overexposed when copied in order to be sufficiently visible.

In connection with the morphological abnormalities provoked by the radiations, some fundamentally different effects obtained in roses must be described here. In April, 1950, some injections of P32 into the stems and branches of about 40 cm high shrubs of roses (variety »Rapture») were made, in co-operation with Mr. SVEN GRÉEN, Director of the Experimental Gardens of the Royal Swedish Academy of Agriculture. The plants were given 2000, 1000, 300, or 100 μC of P32. In the plants, which had received 2000 μC, the leaves *regularly* were of a quite new type: much thicker, with narrower, more roughly serrated leaflets. This leaf form occurred also at the lower doses of P32, but less frequently. Fig. 40 is

an autoradiograph of one leaf from a 2000 μC plant, clearly demonstrating the abnormal leaf shape, and one normal leaf from a plant having received only 300 μC. Among the leaves developed in the late summer, those of abnormal shape grew more rare, and in the following year all treated plants were normal. Obviously the rose variety investigated has an ability to respond to a continuous high intensity of β-radiation through the formation of a new type of leaves.

Similar effects have been obtained with the same agent in potatoes by HAGBERG, NYBOM, and TEDIN (unpubl.) at Svalöf.

DISCUSSION.

The chief purpose of the present investigation was to evaluate if radioactive isotopes can be used in fruit tree breeding (*i. e.* if they cause genetical changes) and, further, to study the most suitable conditions for their application with such purposes. Due to the smallness of the material, no mutations have yet been found; the evaluation has, therefore, to be based on conclusions drawn from other effects.

In the cherry trees used in dose determination experiments (p. 413; Table 4), P32 doses have been reached which obviously have caused the death of the buds. The tolerance dose for survival seems to be in the region of 6000—8000 rep. A direct comparison of this figure with the 5000 rep of X-rays causing a decreased survival of scions cannot be made, due to the very different conditions of radiation application and energy uptake. A great many experiments with seeds of different plant species clearly demonstrate a much higher radiation tolerance in the dormant state (in barley about 5 times; *cf.* EHRENBERG *et al.*, 1952) compared to germinating or growing plants. Scions which have always been irradiated (X-rays) in the dormant state probably do not die because the buds are damaged, but on account of a decreased ability to new callus formation after grafting. The X-ray dose necessary to kill individual dormant buds will probably be higher. In the case of radioisotope treatment most of the radiation occurs in the more sensitive state of swelling and growing.

Also a direct comparison on energy basis of the frequencies of the two main types of morphological changes in the fruit trees is very difficult, already from differences between the units treated: scions, *i. e.* regions of limited and easily defined size in the case of X-ray and neutron irradiation *contra* whole trees or branches, or individual buds in the case of treatment with radioisotopes (the distribution of the latter

can never be exactly known). A comparison based on individual buds having absorbed known amounts of radiation energy would of course be possible, but can at present only be made semi-quantitatively. As it can be stated — through direct measurements or through second-hand conclusions — that in most cases of P32 treatment of trees and branches several buds have absorbed energy quantities of the same magnitude as the X-ray doses given in parallel experiments, Tables 7—11 indicate that the effectivity of P32 for the production of, especially, bifurcations is smaller than that of X-rays. The reason for the difference may be connected with the conditions for the application of the radiation, especially with the prolonged irradiation time, but also with the low specific ionization of P32 β-rays, which may contribute to the smaller effectivity of this agent (*cf.* p. 386). The outstandingly high effectivity of the neutrons, *i. e.* the radiation causing the highest ion density, points in this direction. According to the dosimetry developed by EHRENBERG and NYBOM (1952), 1 dis would equal about 1/25 rep in buds, *i. e.* all the neutron doses applied are in the region 20—200 rep. The low frequency of damage caused by S35, the specific ionization of which is similar to that of X-rays, may be due to the fact that only rather low doses were reached in the experiments here reported (*cf.* Table 4).

The present preliminary investigation clearly demonstrates that it is possible by aid of P32 and S35 to apply radiation doses in buds of a magnitude which from experiments with X-rays is known to give mutations in frequencies perceptible in a limited material (for preliminary notes on X-ray mutations in fruit trees, see GRANHALL, 1949 and 1951). For the purpose of economizing, *i. e.* in order to avoid losses of the expensive isotopes it is extremely important to choose the correct time for treatment, which in any case is not earlier than just after the start of sprouting. Probably it will also be possible to improve the technique of concentrating the injected solution to the upward sap-stream, thus minimizing the losses which occur in the adjacent inert tissues. As the isotopes exert their effects on the tissue in a state which from experiments with barley is known to be more sensitive also from a mutational point of view (*cf.* EHRENBERG *et al.*, 1952) than the dormant state, to which the treatment with X-rays and neutrons is bound, and as it has been proved definitively that the mutagenic effects of the isotopes in barley are qualitatively different from those of X-rays (unpubl.; preliminarily reported by EHRENBERG *et al.*, 1952), the radioisotopes seem to constitute a very valuable complement to X-rays and neutrons

in systematical mutation work in fruit trees. Their value in this respect is still more increased through the fact that the emitted radiation is concentrated to the embryonal parts of the trees, leaving the rest relatively undamaged.

SUMMARY.

(1) For the purpose of obtaining high β-radiation doses in the sensitive vegetation points of young fruit trees different methods of introducing P32 and S35, given as phosphate or sulphate, respectively, into the trees have been compared. Injection with small volumes of concentrated isotope solutions into not only stems and branches but also individual buds or bud bases by the aid of a micrometer syringe after the start of sprouting in the spring has turned out to be a convenient method.

(2) The spreading and distribution of the isotopes in different parts of the trees have been determined through direct analysis or autoradiographs. An accumulation, especially of P32, to meristematical regions is observed.

(3) A method for the determination of tissue doses has been worked out. Doses high enough to cause lethality in individual buds are easily obtained, in any case with P32.

(4) The frequencies per unity dose of the primary biological effects studied (shoot bifurcations and leaf deformations) seem to decrease in the order neutrons $>$ X-rays $>$ P32 β-rays. Probably these differences depend upon the ionization densities of the radiations. In this connection a case of regularly changed leaf shape in roses after P32 treatment is described.

(5) It is concluded that the radioisotopes will be valuable in mutation work with fruit trees.

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THE MODE OF BIVALENT ORIENTATION IN THE HEMIPTERA

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AS was pointed out by THOMSEN (1927) and RIS (1942), the sequence of meiotic divisions is postreductional in Hemiptera Homoptera Sternorrhyncha. The order of divisions is reversed in the group Auchenorrhyncha, where so far the first division is known to be reductional in all cases. In Hemiptera Heteroptera the autosomes undergo a prereductional meiosis in all the species studied in this respect. The sex chromosomes of Heteroptera divide postreductionally with only few exceptions (*e. g., Archimerus calcarator*).

The sequence of meiotic divisions is characteristically different in different systematic groups. The dissimilarity between the sternorrhynchous and the auchenorrhynchous homopterans is phylogenetically significant (see RIS, 1942). The importance of reduction type in Hemiptera systematics is evident. Strangely enough, some interesting exceptions to the uniformity of reduction type within the systematic division have not hitherto been noticed and described.

Prereduction is the consequence of the co-orientation of centromeres in the first meiotic division. Correspondingly, auto-orientation of centromeres in the first spermatocyte or ovocyte makes postreduction possible. (For a theoretical elucidation of these topics, see DARLINGTON, 1937, and OKSALA, 1943, 1948.) The works mentioned show clearly that there are differences in precocity which ultimately determine the mode of orientation in meiosis. The orientation here referred to is that of localized centromeres. In Hemiptera the centromere is known to be diffuse. The possible significance of different centromere types in bivalent orientation is beyond the scope of this article. The peculiar character of the centromere may well be a facilitative factor contributing to the variability of orientation phenomena encountered within Hemiptera species.

The first metaphase bivalents of Heteroptera usually have a rounded outline when seen in polar view. The metaphase plates of some, *e. g.* the coreid bug *Syromastes marginatus* L., are, however, illustrated with dumbbell-shaped chromosomes by some authors (*e. g.*,

GEITLER, 1939). In order to get a better comprehension of bivalent orientation in this species, I have examined preparations including first spermatocyte metaphase plates of several Finnish specimens. Professor ESKO SUOMALAINEN has kindly permitted me to use these preparations made by him from material collected in the neighbourhood of Helsinki.

Often the long and the short axis of late diakinesis tetrads in *Syromastes* cannot easily be discerned or the dividing lines of the chiasmata seen. During late diakinesis or in a first meiotic metaphase plate

1

2

Figs. 1—2. *Syromastes marginatus* ♂. Side (Fig. 1) and polar (Fig. 2) views of first maturation metaphases. The chromosomes form a ring in all the cells. The X_1X_2 chromosome complex lies outside the ring in Fig. 2. The polar view shows two clearly and four less clearly dumbbell-shaped bivalents, all corresponding in their orientation to the two best-focused bivalents of the upper side view in Fig. 1 (auto-orientation). The three round bivalents in the ring in Fig. 2 have an orientation identical with that represented by the bivalents of the lower side view (co-orientation). — Carnoy, Heidenhain, paraffin sections about 15μ thick, $\times$ 1800.

the bivalents gradually acquire a dumbbell shape with their mostly distinct median constrictions indicating the positions of terminalized chiasmata. The long axes of the bivalents may be parallel with the direction of the spindle. In certain of the cells, however, some of the bivalents lie at an angle of 90° to the others. The bivalent halves on the two sides of the chiasma lie in the direction of the metaphase plate and not in the direction of the spindle. The obvious auto-orientation of these bivalents is demonstrated in Figs. 1 and 2. Especially in side views

the 90° inverted positions of these bivalents in relation to their neighbours can be clearly seen. The two types of bivalent orientation are thus represented in one and the same cell.

Among the lygaeid bugs there can be found further peculiarities of bivalent orientation. PFALER-COLLANDER (1941) has concentrated on the comparative karyology of this group, but has not discussed orientation phenomena. She has kindly given me a chance to examine her preparations. These are illustrative in important respects, but a clear view of the orientation phenomena in lygaeid meiosis can already be formed from the microphotographs taken by Dr. PFALER-COLLANDER.

On studying the photographs of the first maturation divisions of males and females, a striking dissimilarity is found between the metaphase plates of the two sexes. In the female the plate is in most cases much broader than in the male. An *Eremocoris erraticus* F. female may have a first maturation division metaphase plate twice as broad as that of a male (PFALER-COLLANDER, Figs. 55 and 57). The phenomenon is not, however, perfectly regular. In the first meiotic division the metaphase plate is of much the same size in male and female *Peritrechus nubilus* FALL. (PFALER-COLLANDER, Figs. 41 and 43). Although plates of ovogenesis cannot always be distinguished by their general size, they are, however, easily recognizable by the peculiar shape of their chromosomes. Among the 65 microphotographs included in the two plates, the 6 photographs of female first metaphases really catch the eye by reason of their strange bivalents. The bivalents in polar view are variously elongated, or dumbbell-shaped or even two-cleft. The fissure between the two halves of a bivalent cannot represent any other formation but a terminalized chiasma. This means auto-orientation. Thus, the mode of orientation is almost absolutely different in the two sexes. In the males co-orientation is decidedly the prevalent mode of bivalent behaviour. In the females no possible exceptions to the rule of auto-orientation can be found. Anyone of the bivalents can orientate differently to two successive generations, if these generations are represented by specimens belonging to different sexes.

Both in Heteroptera males and females a relatively broad spindle and a wide and spacious metaphase plate in the first maturation division are two characteristics often appearing together with auto-orientation. In the females the first metaphase is known to be prolonged. Possibly the qualities of the spindle alter in the first ovocyte prometaphase or metaphase so as to permit auto-orientation of the bivalents. In other words, the precocity of the spindle is perhaps eli-

minated by the long duration of the stages. Hypotheses based on timing relations are, however, of little value for such cases as *Syromastes*, where separate bivalents in the same cell orientate in different ways. We have here one further example of the puzzling changeableness of chromosome behaviour so typical of organisms with a diffuse centromere.

In the Homoptera Auchenorrhyncha one cercopid species studied by me shows signs of ovocyte bivalent auto-orientation, but the case is by no means established. For example, in the euscelid *Athysanus argentatus* F. the bivalents undoubtedly co-orientate in all the eggs inspected. In the males I have found no possible cases of auto-orientation in the meiosis of twenty-two species representing seven families.

The above discussion on the distribution of types of orientation in the Hemiptera can be interpreted in terms of reduction types. Co-orientation is always followed by the breaking of chiasmata in the first maturation division. In the case of auto-orientation the chiasmata are preserved until the second division, where there is only one chromatid on each side of every chiasma. It is just by chiasmata that the double chromatids are kept together during the later prophase stages of meiosis. Owing to the interchange of segments of chromatids, as far as any individual gene is concerned, either of the divisions may, by exchange, be reductional. From the mechanical and cytological point of view the time of the breaking of the chiasmata must be the criterion for separating between pre- and postreduction. Co-orientation is followed by prereduction, auto-orientation by postreduction.

Hemiptera.

Heteroptera.	*Homoptera.*
A. Prereduction.	A. Prereduction.
1. Autosomes in spermatogenesis of most species.	1. All chromosomes in spermatogenesis of species of Auchenorrhyncha.
2. Sex chromosomes of some Coreids.	2. All chromosomes in ovogenesis of *Athysanus argentatus*.
B. Postreduction.	B. Postreduction.
1. All chromosomes in ovogenesis of Lygaeids.	1. All chromosomes in spermato- and ovogenesis of species of Sternorrhyncha.

2. Sex chromosomes in spermatogenesis of most species.
3. Certain chromosomes in spermatogenesis of *Syromastes*.

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SEED DEVELOPMENT AFTER 2x, 4x CROSSES IN GALEOPSIS PUBESCENS

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INTRODUCTION.

RECIPROCAL pollinations between a diploid and its autotetraploid result in aborted seeds, notwithstanding the close relation of the crossed plants. In several cases not a single germinable seed has been produced, in other cases a few seeds; only rarely are such crosses compatible (*Populus, Beta*). Few crosses have been submitted to an embryological-histological investigation of seed development following these pollinations: *Datura Stramonium* (SANSOME, SATINA, BLAKESLEE, 1942), *Lycopersicon pimpinellifolium* (COOPER and BRINK, 1945), *Secale cereale* (HÅKANSSON and ELLERSTRÖM, 1950; LÖWENSTEIN, 1951), *Zea mays* (COOPER, 1951). In $2x \times 4x$ crosses of *Datura* the cause of seed abort is failure of fertilization, diploid pollen tubes bursting in the style of the diploid. In all the other crosses fertilization occurred and seed development was normal for some time; but sooner or later the rate of development slowed down, and disturbances and disintegrations appeared in the endosperm, ultimately leading to the disappearance of this tissue and death of the embryo.

Seed abortion in crosses between species belonging to an alloploid series has been much more often studied. COOPER and BRINK (1945) compared seed development after intra- and interspecific crosses with the same species. They stress that the course of seed failure associated with matings between the diploid species *Lycopersicon pimpinellifolium* and its autotetraploid is very similar to the development after the reciprocal crosses of the latter with the diploid species *L. peruvianum*. These authors (see BRINK and COOPER, 1947) have emphasized the importance of the endosperm in seed development: the death of the embryo in aborting seeds »is a secondary phenomenon originating in abnormal development of other parts of the seed, notably the endosperm». They have also studied certain deviations from the normal of the somatic, maternal tissue of the aborting ovules. The first signs of somatic changes, such as excessive growth of nucellus or integument

tissue, excessive development of the endothelium layer, deficient development of the ways leading nutrients to the endosperm, may appear rather early. Therefore they were considered for some time to be primary, being a cause of the disintegration of the endosperm, and the term »somatoplastic sterility» was coined. Later the changes in the maternal tissue were no longer considered to be primary though the term is still in use, where such changes occur; they are not observed in Gramineae.

Seed abortion after reciprocal autoploid crosses leads MÜNTZING (1930 b) to the conclusion that a fixed quantitative relation between the chromosome numbers of maternal tissue: endosperm: embryo is necessary to a normal seed development. Normally this relation is 2: 3: 2, but after crosses between plants having different number of genomes this relation is changed and is different in reciprocal crosses: in $2x \times 4x$ it is 2: 4: 3; in $4x \times 2x$ it is 4: 5: 3. This may explain the different aspect of seed abortion in reciprocal crosses. However, crosses between species with the same number of chromosomes may also show seed abortion, different after reciprocal pollinations. In such cases the abortion is a result of qualitative differences between the genomes of the crossed species; and owing to the primary endosperm nucleus being formed of two female and one male nucleus its genic constitution is different after reciprocal crosses.

MATERIAL.

In the present paper the seed development after reciprocal crosses between diploid and autotetraploid *Galeopsis pubescens* is described. I have received the material from Professor A. MÜNTZING, who has in cultivation the species of the *Tetrahit* section of *Galeopsis,* that is the diploid species ($2n = 16$) *speciosa* and *pubescens* and the tetraploid species ($2n = 32$) *Tetrahit* and *bifida.* MÜNTZING has shown the close relation between these species, among other things through his well-known synthesis of *G. Tetrahit* from the two diploid species. Crosses between species with the same chromosome number are successful, but crosses between a diploid and a tetraploid do not result in germinable seeds. When the diploid species is the mother plant there is no swelling of the ovaries, presumably owing to failure of fertilization. After tetraploid $\times$ diploid crosses the ovaries increase in size and contain after five days ovules with normal endosperm and embryo; after fourteen days, however, degeneration of the endosperm and embryo

is evident (MÜNTZING, 1930 a). Similar is the behaviour of synthetic *G. Tetrahit* in crosses with *G. pubescens* and *G. speciosa.* When the tetraploid is mother plant fertilization occurs, development of endosperm and embryo is normal at first, but ends in degeneration, the turning-point presumably being 10—12 days after fertilization. In the reciprocal cross »erfolgte dagegen nicht die geringste Anschwellung der Ovarien. Offenbar liegen hier zwei verschiedene Phänomene vor» (MÜNTZING, 1930 b).

The tetraploid form of *G. pubescens* has been produced experimentally through colchicine (MÜNTZING and RUNQUIST, 1939). It is a typical autotetraploid with several giant characteristics (MÜNTZING, 1941). The properties of tetraploid *G. pubescens* have been thoroughly studied by EKDAHL (1949), who also has produced figures illustrating the high seed sterility of this autotetraploid. While the diploid strain had 2,35 seeds per flower, the tetraploid had only 0,22 seeds per flower. MÜNTZING (1941) has shown that autotetraploid *pubescens* and *speciosa* are completely incompatible with their original diploid forms, both reciprocal crosses failing to give any seeds. Octoploids have been produced from the tetraploid species.

In his paper »Beiträge zur Kenntnis der Samenentwicklung der Labiaten» SCHNARF (1917) described seed development in *Galeopsis.* He had investigated the four species of the *Tetrahit* section and also *G. Ladanum* and *G. angustifolia.* The observations made during my own investigations agree on the main points with the previous description. A short resumé may precede the description of the development in the crosses.

The embryosac consists of a larger and wider part containing the egg-apparatus and a smaller, narrow part with the central nucleus (the secondary embryosac nucleus) and the antipodals (Fig. 1). The former is called the upper, the latter the lower or endospermal chamber. »Upper» and »lower» are inadequate terms, because the micropyle of the ovule is turned downwards, but the terms are used in conformity with SCHNARF. The nucellus of the ovule has disappeared during the development of the embryosac, which now lies in the integument. The innermost layer of the integument surrounding the lower chamber has taken on the appearance of an endothelium. The upper chamber is not limited by an endothelium; and its boundary against the integument tissue is less clear-cut. Below the embryosac a so-called hypostase is formed in the chalaza. The hypostase consists of small, empty cells with thick, lignified walls; it is also near the

end of the vascular bundle which supplies the endosperm and the ovule with nutrients; here is probably the main way of the nutrients to the growing endosperm and embryo.

The endosperm and the embryo are formed in the lower chamber of the embryosac. Endosperm formation starts with the fertilized central nucleus (the primary endosperm nucleus) approaching the antipodals and dividing. A small basal endosperm cell is formed which later becomes binucleate but does not take part in the further development of the endosperm. In certain Labiatae the basal cell grows considerably and is a chalazal haustorium; this, however, is not the case in *Galeopsis*. The large endosperm cell is divided longitudinally into four cells: they are very long, reaching into the lower part of the upper chamber, but open towards the interior of this chamber (*cf.* Fig. 2). Transverse walls then divide the long cells into several stories of short cells. The cells of the lowest and of the uppermost storey do not partake in the formation of the bulk of the endosperm. The four cells of the lowest storey are elongated, their number increases to eight, and they grow somewhat in the direction of the chalaza, surrounding as a tube the basal endosperm cell. The behaviour of the four cells of the uppermost storey is rather strange. The nucleus passes to the part of each cell which is in the basal section of the upper chamber. Later, the nuclei move further, forming a group when they have arrived in the region of the chamber which is not divided into separate cells. The nuclei show increased size and have large, often irregularly formed nucleoles (*cf.* Fig. 8). They lie in a large mass of cytoplasm which takes up a position at the upper wall. Here the cytoplasm and the nuclei penetrate the wall of the upper chamber, making an open canal and passing into the room of the ovary (Fig. 10 *b*). The movement of this micropylar haustorium is now not an active growth but rather a passive flow, which stops near the base of the ovary where the nuclei lie against the wall (the endocarp).

The lower endospermal chamber has a curvature which becomes more pronounced during the early development. Here the young endosperm is broader. The upper chamber is united with the endosperm tissue through the »isthmus», a very narrow neck which earlier contained the endosperm cells the nuclei of which had moved into the upper chamber, but soon is closed (Fig. 9). The embryo develops into the endospermal chamber. The fertilized eggcell immediately begins to grow in the direction of the lower chamber, its apical end

penetrating through the narrow entrance into that chamber, and the embryo is surrounded by endosperm cells (Fig. 8).

SCHNARF found only insignificant differences between the species of the *Tetrahit* section. It is true that the basal binucleate endosperm cell was only observed in *G. bifida* but SCHNARF was convinced of its occurrence in the other species, too. He has investigated a number of genera of Labiatae. The initial stages of endosperm formation and the appearance of the endosperm haustoria is different: different types are discerned. The most primitive condition is found in genera lacking any haustorial differentiation of the endosperm (*Scutellaria*); more advanced is the endosperm of *Ajuga,* which has a rather small upper and no lower haustorium; a third stage show genera with an upper and a lower haustorium, both of them well developed; while in *Galeopsis* and some other genera a still more advanced development is found, the lower haustorium being reduced, the upper one being very large. The highest specialization is found in *Galeopsis*, the upper haustorium forming an extra-ovular outgrowth.

Seed development after artificial pollinations is the theme of this paper. Emasculated flowers have been pollinated and ovaries fixed five hours, and one, two, four, six, twelve days after pollination. Investigated combinations are $2x \times 2x$, $4x \times 4x$, $2x \times 4x$, and $4x \times 2x$. Fixations were made in 1950 and 1951; from the two reciprocal crosses also in 1948. As a rule, the strain P—A has been investigated. The often poor result of the artificial pollinations made an investigation of open-pollinated ovaries of the diploid and tetraploid desirable. They were fixed one and four days after the beginning of flowering. Each time fixations were made from four plants, the fixations always being kept apart.

OBSERVATIONS.

2x × 2x.

The early collections five hours after pollination were of no value. pollen tubes had not reached the ovules. In this as well as in the other crosses fertilization occurs later than after five hours.

After 1 day most ovules were fertilized. The development after fertilization had been very rapid. The most advanced ovules had 30 endosperm cells, the average number being about 20. At the lowest end of the embryosac a basal, sometimes two-nucleate, endosperm cell is observed and above this four elongated endosperm cells. Al-

ready there may be four endosperm nuclei in the basal part of the upper chamber, or they may be observed during their passage into that chamber. In view of this high number of endosperm cells a comparatively short time after fertilization it is not surprising that it is often laborious to find all stages of the initial development of cellular endosperms. The fertilized eggcell was elongating, its apical end had reached the entrance of the endosperm chamber or, in the most advanced ovules, penetrated into the endosperm. The pollen tube was still visible outside and in the micropylar canal. It is very thick, a fact already observed by HOFMEISTER in 1858. In the material collected in 1950 the number of endosperm cells was lower than in the following year, no ovule showing endosperm nuclei in the upper chamber. A rather high percentage of the ovules were unfertilized also in the open-pollinated flowers.

After 2 days the changes have not been very great. The growth of the ovules has been rather moderate as is clear from the size of fertilized, compared with unfertilized, ovules. The pollen tube outside the micropyle has been resorbed. The four endosperm nuclei in the upper chamber now form a group which has moved across the chamber to the upper wall. An opening in the wall had sometimes been made, the free nuclei making an exit into the room of the ovary. The number of endosperm cells may be about 100, but is often lower. The endosperm is more broad below the isthmus and narrows in the direction of the chalaza. The endothelium cells are flattened or destroyed around the broad part of the endosperm. The largest embryo observed had eleven cells.

After 4 days the chalaza-directed part of the endosperm may have widened and the whole endothelium disappeared. In the majority of the ovules the widening of the lower endosperm is, however, still not initiated. A certain differentiation of the endosperm tissue has begun which is more distinct after six days. An outermost layer of rather small cells has been formed; they are rich in cytoplasm, and form a sort of meristeme. Numerous tangential and radial divisions are observed in this layer. Divisions are also observed in the inner parts of the endosperm but the growth of the endosperm is often less than the growth of the ovule, the endosperm tissue being rather loosely connected with the somatic tissue of the ovule. A cavity is often formed beside or below the endosperm.

The nutrient-supplying organs of the endosperm are now differentiated. Eight endosperm cells have grown a short way in the di-

rection of the chalaza and surround as a tube the basal endosperm cell in the manner described by SCHNARF. The cells are enlarged and have large nuclei. A few times prophase was observed in such a nucleus (Fig. 3). Once about 48 chromosomes were counted. No doubt endomitosis was the cause of the doubling, a process likely to occur in enlarged nuclei of cells with a nutritive function. The endosperm nuclei of the upper chamber now, as a rule, have their position outside the ovule at the ovarial wall. Already before leaving the chamber they showed increased size and large nucleoles; now they are still larger but never show prophase structure. In a number of ovules the four endosperm nuclei in the upper chamber remain rather small and degenerate within the chamber. Such ovules usually have a less advanced endosperm, which probably later degenerates. The endosperm tissue near the isthmus consists of rather large cells. These cells seem to have a special function as they are never filled with storage. One or two of them are very large and contain a large nucleus with a changed content, somewhat similar to a haustorial nucleus. The embryo has in many ovules 20—30 cells, but often more. As the embryo is often seen in cross-section its size is estimated by counting the number of cells occupying its diameter. They may be six. The largest diameter of the endosperm may be ten cells, and, as the endosperm cells are larger, this tissue seems considerably broader than the embryo.

After 6 days the endosperm is much enlarged, but as the volume of the integument tissue has increased still more, a large cavity is present in most seeds (Fig. 16). The diameter of the embryo is eight—twelve cells. It is elongated but completely undifferentiated. Some seeds were smaller, being at the two- or four-day stage.

After 12 days seed development is near its end. Later than 6 days the endosperm tissue grows and fills the larger part of the ovule, destroying the integument tissue, of which only a few layers may be left. The outer part of the endosperms has rather small cells while the interior is occupied by very large cells having irregular form. The nuclei of these large cells are small. This may depend on the fact that these cells have no function; they are ephemeral, being destroyed by the growing embryo. The period of rapid growth of endosperm is succeeded by a period of rapid embryo growth. Now after 12 days the embryo may occupy $^3/_4$ af the length of the seed and has large cotyledons and a primary root. The remaining endosperm tissue contains storage, except for the region near the isthmus. The upper

chamber is still visible as well as the hypostase with remains of the basal endosperm cell.

Small seeds having endosperm and embryo at the six-day stage were found. Thus, seed development may in exceptional cases be slow or interrupted. In fact, a few ovules with degenerating endosperm have been found during the investigations.

4x × 4x.

The *gigas* form of *Galeopsis pubescens* has larger ovules, larger embryosacs and larger cells and nuclei in the embryosac than the normal diploid form. However, a certain qualitative difference is also indicated. The embryosac may contain two polar nuclei in close contact instead of a central nucleus. Failure of fusion of polar nuclei was very frequent in the material collected in 1950, but more exceptional in 1948 and 1951. In 1950 about 40 % of the unfertilized ovules in $4x \times 4x$ and $4x \times 2x$ crosses had polar nuclei. The cause of this difference is perhaps due to external factors.

Many ovules lack a normal embryosac owing to different causes. The embryosac may degenerate at an early stage of development; the development may be belated, an ovule one day after pollination having a two- or four-nucleate embryosac; the embryosac may be irregular. In irregular embryosacs cell-formation was incomplete or had failed, free nuclei often forming a group with the two polar nuclei (»supernumerary polar nuclei»). As a rule, such free nuclei are in the upper chamber; an exception was an embryosac with five nuclei at the bottom of the upper chamber, but one large nucleus in the lower chamber above the chalaza. Exceptional ovules with a very reduced upper chamber and an enlarged lower chamber with a central nucleus were observed. A large majority of the ovules have, however, a normally organized embryosac. Thus, so-called haplontic sterility plays a certain, though only minor, rôle. The most important cause of the large seed sterility of autotetraploid *Galeopsis pubescens* is no doubt failure of fertilization.

Ovules in the ovaries of the open-pollinated flowers were very often unfertilized. After artificial pollination the number of unfertilized tetraploid ovules was still higher, many fixations not showing a single ovule with endosperm. One day after the artificial pollination fertilized ovules contained only few endosperm cells, the highest number observed being nine. The eggcell was elongating, but had not reached the endosperm chamber. Free nuclei never occurred in the upper

chamber. Thus development was less advanced than in the diploid: slower growth of the pollen tube may have contributed to this. However, in the open-pollinated ovaries the difference was less striking.

Two days after pollination the ovule may contain as many as eighty-five endosperm cells, though usually a lower number. A typical endosperm is shown in Fig. 8. At the chalaza end the three antipodals are observed. They have the same appearance as in the diploid but are larger and more persistent. A small basal endosperm cell is seen, above, in all eight elongated cells (only four are designed), then a storey of small endosperm cells, higher up larger cells in the widest part of the endosperm chamber, and then smaller cells again. This structure of the endosperm shows that it is similar in tetraploid and diploid *G. pubescens*. The free endosperm nuclei in the upper chamber have increased in size; they have not moved to the upper part of the chamber but form a group near the wall near the base of the chamber. The embryo consists of two cells. Also other ovules showed the formation of the canal near the bottom of the upper chamber. The largest embryo had four cells. Most of the endothelium has disappeared, it is only present around the lowest part of the endosperm.

After 4 days the more rapid-growing ovules had considerably increased in size. The volume of the endosperm is more variable than in the diploid. A difference is the behaviour of the micropylar haustorium. Much more often than in the diploid no extra ovular outgrowth is formed. In such ovules only a rather small mass of cytoplasm surrounds the nuclei, which degenerate in the chamber or sometimes in the canal initiated in the wall of the chamber. The micropylar endosperm haustorium is, thus, less developed than in the diploid. When an extra ovular outgrowth is formed the opening in the wall is not formed at the upper wall but near the base. The endosperm tissue is in its broadest part five to ten cells across. In the chalaza-directed part widening rarely has begun. A fairly developed embryo had 22 cells.

After 6 days the impression is formed that the development of the endosperm is somewhat less rapid and the young endosperm less differentiated than in the diploid. The elongated cells near the chalaza are in most cases small but may show in the largest ovules increased size and large nuclei with a changed content. In the cases where an extra-ovular outgrowth has been formed, its nuclei are smaller than in the diploid. The upper chamber is now also smaller, and the bottom is often pushed against the upper wall giving the chamber the ap-

pearance of an umbrella or a mushroom. In this connection the isthmus becomes longer than in the diploid. A special differentiation of the endosperm tissue in the vicinity of the isthmus may also be observed in the tetraploid but very large cells were rarely seen. The outermost endosperm layer is less regular than in the diploid, consisting of cells of different size. The embryo is in the best ovules comparable in size with diploid embryos, having a diameter of seven to twelve cells.

The size of endosperm and embryo is very variable, more than in the diploid, and ovules with dead endosperm may be found. Collections made in 1950, ten days after pollination, proved to be a good material for observation as to the different aspects of the hexaploid endosperm. In the good seeds endosperm tissue filled the larger part of the interior, but the embryo was still undifferentiated. However, many seeds had a deficient endosperm. It had attained the stage one finds after four days or in weakly developed ovules after six days. This endosperm had now a sickly appearance or was degenerating; the narrow part of the endosperm having connection with the chalaza had disappeared. Thus, rather early the connection with the hypostase and the nutrients from the vascular bundle may be interrupted. In such cases further development of endosperm or embryo is probably prevented. Such smaller seeds have a large cavity near the endosperm. The normal developing endosperms confirm the impression of more slow growth and less pronounced differentiation gained from the earlier stages.

Exceptions to this occur, however: after 12 days a large seed contained an anormally long embryo with cotyledons and primary root, remaining endosperm layers being filled with storage.

Thus the poor seed fertility of tetraploid *Galeopsis pubescens* has different causes: absence of a normal embryosac in the ovule, failure of fertilization, and arrested development after fertilization. A cause of the increased post-fertilization mortality may be aneuploidy of endosperm and embryo resulting from irregularities in the autotetraploid meiosis, but is perhaps more often evidence of an inherent weakness of ovules of the tetraploid, owing to particularities in their structure. A peculiar trait is the weakness of the micropylar endosperm haustorium.

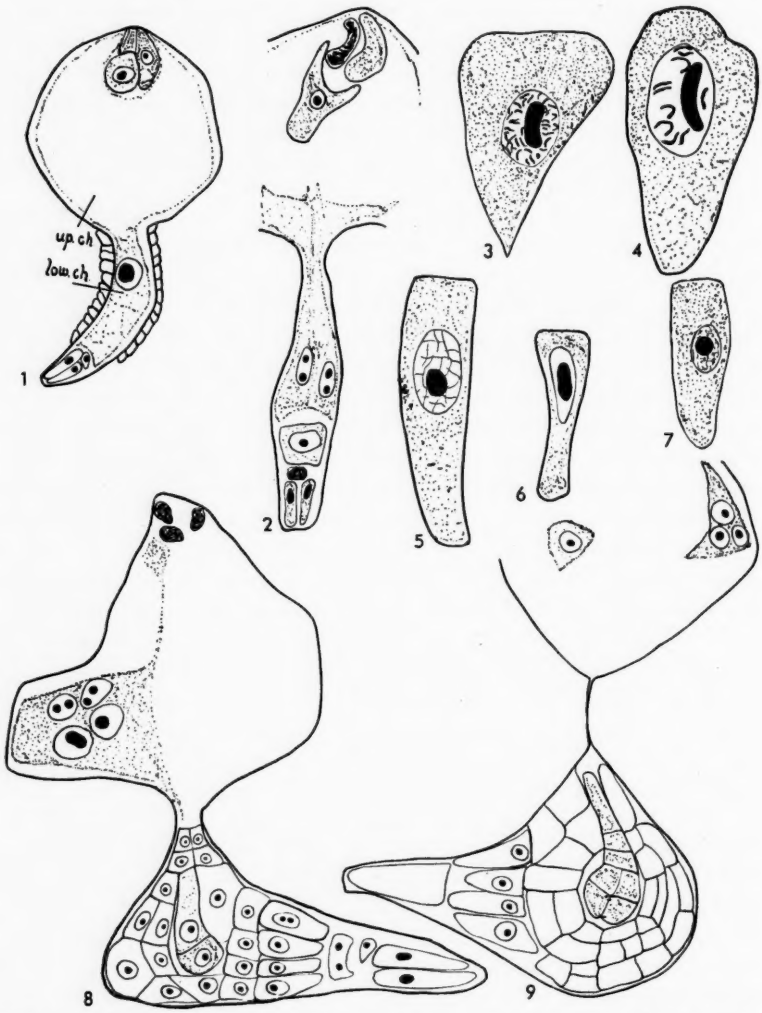
2x × 4x.

Fertilizations occurred all three years but their number was small; judging from the results in 1951, they were, however, as fre-

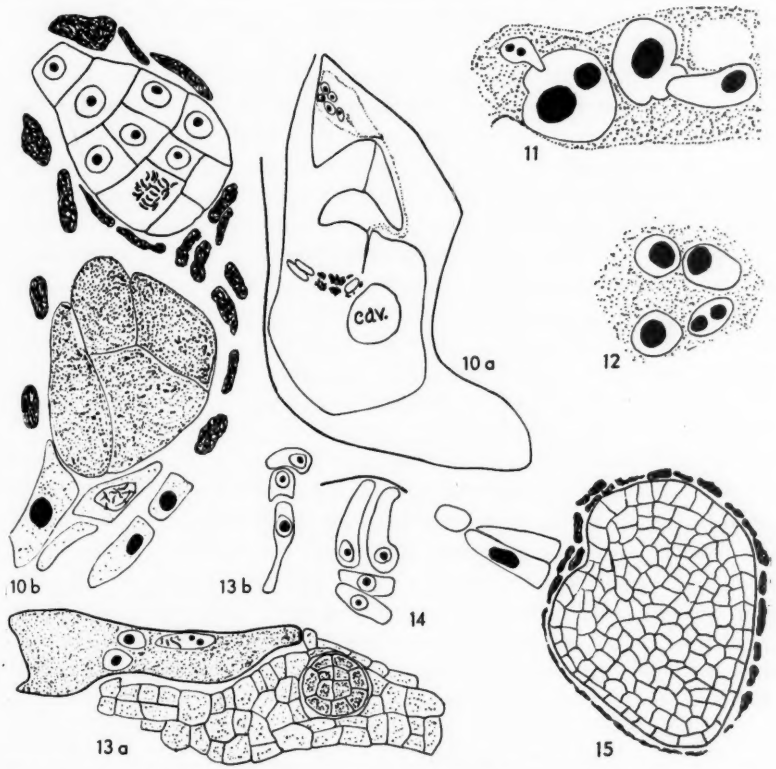
quent as in $4x \times 4x$. After 1 day the number of endosperm cells was higher than in $4x \times 4x$, but less than in $2x \times 2x$. The highest number observed was sixteen. Only one ovule had endosperm nuclei in the upper chamber. A basal endosperm cell, overlayed by four elongated cells, showed that the initial development of the endosperm is normal. The eggcell was elongating but had only once penetrated into the endosperm chamber.

After 2 days there were 30—41 (in 1951) or as many as 70 (in 1950) endosperm cells. The cells were poor in cytoplasm, the eight elongated cells being an exception to this. The endosperm nuclei in the upper chamber were large, had large nucleoles and were surrounded by much cytoplasm. The nuclei have moved from the base of the chamber towards the upper wall although only exceptionally penetrating through the wall. Once only two very large nuclei were observed. SCHNARF has, however, already found two nuclei in some *Galeopsis* ovules. Embryo-formation had often been initiated. Most endothelium cells were flattened or had disappeared, but they were unchanged around the narrow chalaza-directed part of the endosperm.

After 4 days the ovules are considerably larger, Figs. 10 *a* and *b* show a rather typical ovule. The endosperm consisted of comparably few cells, most of them being opaque and having an indistinct nucleus. Such cells no doubt are degenerated. The elongated cells near the hypostase are small and have not grown in the direction of the hypostase; thus, they do not form a tube around the basal endosperm cell. From the upper chamber an extra-ovular outgrowth has been made, the enlarged endosperm nuclei being seen near the base of the ovary. The embryo is vital; it consisted of 22 cells, one of them dividing (Fig. 10 *b*). — Thus, the endosperm had increased in size only insignificantly during two days and in most seeds was degenerating. Still living endosperms are small, no divisions are observed, and further development does not occur. The elongated cells near the chalaza are considerably smaller than in the diploid (Fig. 6), the contact of the endosperm with the chalaza being very weak. The endosperm tissue shows no differentiation, all cells being very vacuolate. However, the micropylar haustorium is well developed and in most endosperm-containing ovules shows an extra-ovular outgrowth with large nuclei often having an irregular form (Fig. 11). There is sometimes more than four nuclei in the outgrowth. How this increase in number has been brought about could not be ascertained. Perhaps a sort of amitosis or a local breakdown of the nuclear membrane



Figs. 1—9. *Galeopsis pubescens.* — *1:* embryosac; up. ch. = upper chamber, low. ch. = lower chamber. × 230. — *2:* $4x \times 2x$, 1 day; three-celled endosperm and elongating eggcell. × 230. — *3—7:* elongated cells of the lowest endosperm storey, 4 or 6 days after pollination, *3* of $2x \times 2x$, *4* and *5* of $4x \times 4x$, *6* of $2x \times 4x$, *7* of $4x \times 2x$. × 700. — *8*: $4x \times 4x$, 2 days; endosperm and two-celled embryo, nearest the chalaza are the antipodals and the basal binucleate endosperm cell. The forming of an outgrowth is initiated in the upper chamber. × 230. — *9*: $4x \times 2x$, 2 days; endosperm and embryo. × 230.



Figs. 10—15. *Galeopsis pubescens.* — *10 a:* $2x \times 4x$, 4 days; ovule with extra-ovular endospermal haustorium, the endosperm itself is already degenerated. cav = cavity formed in the integument. × 50. — *10 b*: endosperm and embryo of this ovule. × 700. — *11*: $2x \times 4x$; nuclei of the extra-ovular endospermal outgrowth. × 700. — *12*: $4x \times 2x$, endosperm nuclei in the upper chamber. × 700. *13*: $4x \times 2x$, 4 days; abnormal development; *a*: endosperm with a curious haustorium and embryo, *b*: the content of the upper chamber. × 230. — *14*: $4x \times 2x$, abnormal organization of the content of the upper chamber. × 230. — *15*: $4x \times 2x$, 12 days. Embryo surrounded by degenerated endosperm, two normal cells. × 700.

had occurred. It may seem strange that the presence of haustoria does not prevent or delay endosperm degeneration. A closer investigation shows, however, a weakness of the micropylar haustorium. It contains little cytoplasm, and often lacks the broad contact surface with the ovarial wall usually formed by the outgrowth in the diploid. Cytoplasm and nuclei are in most cases assembled in the bottom of the

ovary, on account of which a haustorial function seems improbable here.

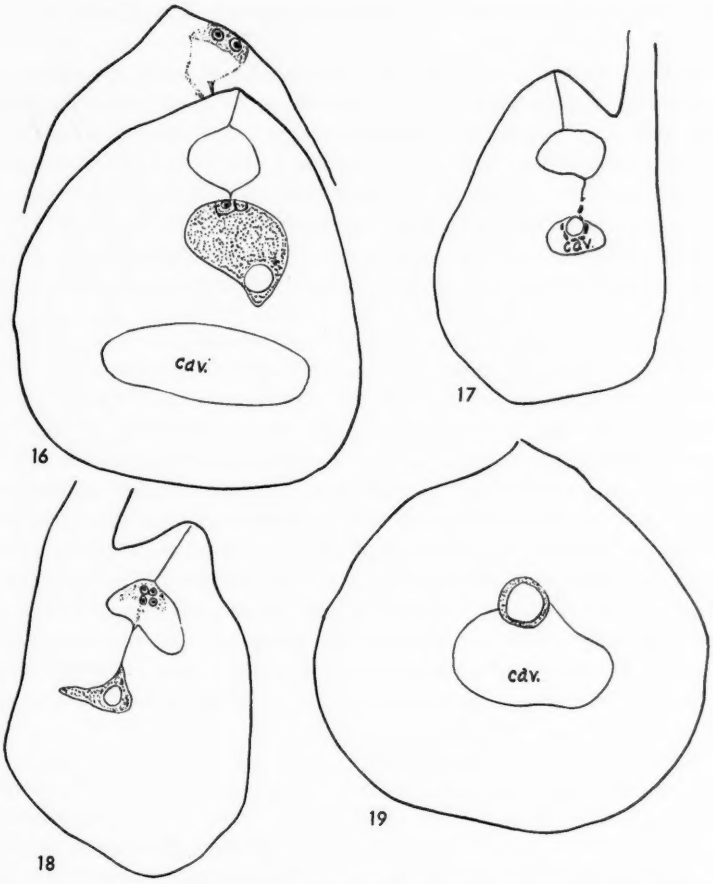
After 6 days the endosperm has completely disappeared in most ovules. In other ovules there are dark-stained rests surrounding the embryo; sometimes a few clear cells with a nucleus are left. The ovules have grown and are two to three times the length of unfertilized ovules. The ovules contain a cavity, which is formed through the growth of the integument tissue and the disappearance of the endosperm (Fig. 17). Only rarely is the embryo also absent, most fertilized ovules contain an embryo of varying size; it may consist of as few as four cells, but often their number is about 20. In one collection a larger tissue of 60 to more than 100 cells was left in the ovule, the largest tissue not having the form or appearance of the embryo. It was uncertain if it was an abnormal embryo or endosperm tissue. The embryo cells are large and were never observed dividing. An instance of quite abnormal development showed an ovule which had fifteen cells in the endospermal chamber and one nucleus in the upper chamber. — After 12 days no seeds contain endosperm or embryo.

The growth of a tetraploid endosperm in a diploid ovule begins normally, but is interrupted very early; degeneration follows rapidly, being often evident four days after pollination. Notwithstanding the weakness of the endosperm the micropylar haustorium is well developed, forming an extra-ovular outgrowth. Embryo development seems interrupted through the endosperm degeneration, but the embryo is more long-lived. The growth of the ovule is fairly normal up to four days after pollination.

4x × 2x.

Here a considerably higher number of fertilizations occurred than in $2x \times 4x$ and in $4x \times 4x$. Development after fertilization proceeded with very variable rapidity. One day after pollination only initial stages of endosperm development were observed in 1948. Fig. 2 shows the basal cell and longitudinal division of the large endosperm cell; the elongation of the eggcell has been initiated. In 1950 there were as many as twelve endosperm cells, the eggcell having grown to the entrance of the lower chamber, while in 1951 the highest number of cells was 21 (most ovules had a lower number), free nuclei being in the upper chamber in more advanced ovules, the end of the eggcell being in the endosperm chamber.

After 2 days a few ovules showed a rapid development. Fig. 9



Figs. 16—19. *Galeopsis pubescens.* — *16*: $2x \times 2x$, 6 days; ovule with outgrowth from the upper chamber, the endosperm is rather large. — *17*: $2x \times 4x$, 6 days; ovule with completely degenerated endosperm. — *18*: $4x \times 2x$, 6 days; ovule, no outgrowth has been formed, the endosperm is small. — *19*: $4x \times 2x$, 12 days; the embryo has grown somewhat at the expense of the small endosperm. — Apparently living endosperm tissue is dotted; it surrounds the spherical embryo. × 50.

shows an ovule from 1948 with a rather large embryo with nine cells. At the chalaza end of the endosperm eight elongated cells are observed, the upper chamber containing three nuclei near the wall and one in the centre. In 1950 the best ovule had 80 endosperm cells

and a three-celled embryo, whereas in 1951 the largest embryo was still more advanced.

After 4 days the peculiar traits in the development are clear. An extra-ovular outgrowth is never formed. Only sometimes the endosperm nuclei of the upper chamber show increased size and increased nucleolar substances; and in certain cases the formation of a canal through the wall is initiated. As in the tetraploid this occurs near the bottom of the chamber; the epidermis is, however, not broken through, and the nuclei have been observed degenerating in the canal. The elongated cells at the chalaza end are small (Fig. 7) but sometimes show a size and structure similar to that in the hexaploid endosperm. There are no very large endosperm cells near the isthmus, though a group of cells show a moderate enlargement. The endosperm tissue is considerably smaller than in the diploid and the tetraploid; its size varies greatly, ranging from 30 cells to 130 cells. Some endosperms show many mitosis figures indicating rapid development; others seem to show interrupted development, but no case of degenerating endosperm was found. A curious endosperm is shown in Fig. 13 *a*. Parallel with the endosperm tissue and growing in the direction of the chalaza is a very large haustorial cell with three nuclei; the upper chamber had three small cells at the place of the egg-apparatus (Fig. 13 *b*), of which none could be the ordinary eggcell because the endosperm contained an embryo. The development of the embryo is rapid. The number of cells occupying the diameter being five to seven (once nine).

After 6 days the normal growth of the embryo and the slow growth of the endosperm are evident (Fig. 18). In larger ovules the embryo shows eight to twelve cells in the diameter whereas the endosperm is always small, one or two layers separating the embryo from the integument tissue (the layers of endosperm cells nearest the embryo have been destroyed). Mitosis in the embryo but not in the endosperm is often observed. The development of the latter has stopped but degeneration has, as a rule, not set in. The differentiation within the endosperm is deficient: outer layers are not more rich in cytoplasm; there are no very large cells near the isthmus; only rarely the elongated cells are large and have nuclei with changed content. The upper chamber is always small and has no opening. The ovules are considerably smaller than after fertilization with haploid pollen tubes. This slow growth explains why cavity formation, notwithstanding the

small volume of the endosperm, is rarely observed. Many ovules show a less advanced development.

After 12 days the endosperm has partly or wholly degenerated and disappeared. Fig. 19 shows one or two layers around an embryo which has a diameter of twelve cells, that is, it has not been much enlarged during six days. The ovule has increased in size, and large cavities are in the integument tissue. The largest embryo found is shown in Fig. 15. It has taken the elongated form (it is cross-sectioned) but is undifferentiated. It is surrounded by degenerated endosperm layers, cellular structure is however shown in the direction of the chalaza.

Here an anomaly may be described concerning a fertilized ovule with normal endosperm and embryo. Near the earlier place of the egg-apparatus were four cells (Fig. 14). It seemed, judging from the form of the cells, to be two synergids and an aberrant, two-celled embryo coming from an eggcell which had not undergone any elongation. I am, however, inclined to interpret the nuclei of the cells as the four endosperm nuclei which have remained small and have been enclosed in cells. A few times small cells, instead of nuclei, have been seen in the upper chamber and, thus, if such cells are formed near the egg-apparatus the normal formative influences still seem to be active. — A cleavage of the cytoplasm between the separated polar nuclei was observed in an unfertilized embryosac.

In $4x \times 2x$ endosperm and embryo attain larger sizes than in $2x \times 4x$. Development commences normally but endosperm slows down after four days and a tissue of very reduced size is formed, its growth being stopped after six days. The embryo grows better than the endosperm but it does not attain the differentiated stage. The micropylar endospermal haustorium is very weak and does not form any extra-ovular outgrowth.

CONCLUSIONS.

An important factor causing seed sterility in *Galeopsis* is failure of fertilization. EKDAHL (1949) counted the seeds in 2347 flowers from five plants of diploid *G. pubescens*; he found only 2,35 seeds per flower. Investigation of open-pollinated and artificially pollinated ovaries shows unfertilized ovules. Tetraploid *G. pubescens* has a very high percentage of unfertilized ovules. In $4x \times 2x$ and $2x \times 4x$ crosses the great majority of the ovules were unfertilized; however, in

$4x \times 2x$ the result of artificial pollinations was better than in $4x \times 4x$ and $2x \times 4x$. There is apparently a difference in the function of haploid and diploid pollen tubes, haploid tubes functioning better than diploid tubes in diploid and in tetraploid stylar tissue. This conforms with expectation. JULÉN (1950) measured the speed of pollen tubes in the styles of tetraploid *Trifolium pratense*; the speed of haploid tubes was 60 μ, of diploid tubes only 35 μ per minute.

The histological aspect of post-fertilization disturbances during seed development in angiosperms is different owing to differences in size and structure of ovule and endosperm, rate of development, and so on; it is, for instance, different in a sympetaleous and a grass species. *Galeopsis* has, like all true Sympetalae, an ovule with ephemeral nucellus and one thick integument, and, as many members of the order Personatae of VON WETTSTEIN or Tubiflorae of ENGLER, *ab initio* cellular endosperm formation, early formation of a micropylar and a chalazal endosperm haustorium, and elongation of the fertilized eggcell to bring the developing embryo in contact with the endosperm. Previously two cases of reciprocal autoploid crosses with a similar embryology have been investigated: *Datura Stramonium* (SANSOME, SATINA and BLAKESLEE, 1942) and *Lycopersicon pimpinellifolium* (COOPER and BRINK, 1945). These species have *ab initio* cellular endosperm formation, although they lack haustoria.

The tetraploid *Datura* has a high seed sterility owing partly to failure of fertilization, partly to zygotic sterility. The $2x \times 4x$ cross succeeds if the mother plant is a diploid with tetraploid transmitting tissue in the style through which the tubes grow. Fertilizations occur after 24 hours, the rate of endosperm formation in the four combinations being the same up to the division of the eggcell when 15—20 endosperm cells have been formed. Then the rate in $2x \times 4x$ and $4x \times 2x$ slows down; after four to six days when the endosperm contains 50 to 80, the embryo 2 to 6 cells, disintegration of the content of the embryosac begins, rather simultaneously in the reciprocal crosses and proceeding very rapidly. Exceptionally a more normal development results in good seeds, in $4x \times 2x$ 0,5 % were good, but also in $2x \times 4x$ germinable seeds were obtained, although still more rarely.

The seed sterility of the autotetraploid form of *Lycopersicon pimpinellifolium* is ascribed to restricted pollen tube growth, less to the breakdown of seeds during development. Decreased rate of endosperm growth in both the reciprocal crosses is evident. After four days the eggcell was always undivided and in $2x \times 2x$ the number of

endosperm cells was on an average about 8, in $2x \times 4x$ 4, in $4x \times 2x$ 3,5 and in $4x \times 4x$ 2,5. After six days the numbers were 148, 28, 20 and 36, respectively, the number of embryo cells being 8, 4, 4 and 2. Shortly after six days $2x \times 4x$ seeds collapse. $4x \times 2x$ seeds develop further, although very slowly, having after eight days 55 endosperm nuclei as against 512 (!) in $4x \times 4x$ seeds; after sixteen days $4x \times 2x$ endosperms have disappeared; the embryo also grows slowly and is very small and starved. Normal endosperms show during development a certain differentiation, the cells at the chalaza end and the outermost layer having a dense cytoplasm. The small endosperms in $2x \times 4x$ and $4x \times 2x$ show no differentiation, all cells being large and highly vacuolate. This lack of differentiation in weak endosperms was also observed in *Galeopsis*.

In *Galeopsis* fertilization occurred much earlier than in *Datura* and *Lycoperiscon*. The initial development of the endosperm in *Galeopsis* is incomparably more rapid. After one day the *Galeopsis* endosperm has more cells than the endosperm of *Lycopersicon* after four days. The most rapid development in *Galeopsis* is shown in $2x \times 2x$. The low number of fertilizations in $4x \times 4x$ and $2x \times 4x$ make comparisons dangerous. The slides showed a slower development in $4x \times 4x$ than in the two reciprocal combinations after one day, but after two days no differences seem to occur. There is a pronounced difference between endosperm development in $2x \times 4x$ and $4x \times 2x$. In the former the development stops very early and degeneration sets in rapidly; it is evident after four days. In $4x \times 2x$ endosperm development continues, although at a very slow rate; after 6 days no trace of degeneration is seen; but after 12 days only remnants of the endosperm are left. The rate of development of the *Galeopsis* embryo is slower than the endosperm development; it has not been studied in detail. The rather rapid development in $4x \times 2x$ is striking: up to six days it is as rapid, or more rapid, than in $4x \times 4x$. Thus, the embryo in the former cross is large compared with the small endosperm tissue, but further growth is inconsiderable and the differentiation stage is not attained. The arrest of embryo growth seems to be secondary to the endosperm degeneration. At any rate, the embryo is more long-lived than the endosperm, its growth continuing longer. In $2x \times 4x$ and $4x \times 2x$ crosses with *Galeopsis pubescens* no germinable seeds have been obtained. Development does not even attain the stage of the endosperm seriously encroaching on the integument tissue, still less the following stage of rapid embryo growth. Seed development in

the fixed material of $4x \times 2x$ seems to be worse than in synthetic *Tetrahit* $\times$ *pubescens* where MÜNTZING estimated the turning-point of development to be 10—12 days after pollination.

FAGERLIND (1948) found that in pseudo-incompatible species crosses in the genus *Rosa* the balance between endosperm and embryo is very labile, disturbances of the fixed synchronization in the development of maternal tissue of the ovule, endosperm and embryo being very frequent. This lack of synchronization he considers to be the cause of the seed abortion. Lack of synchronization in the development of endosperm, embryo and maternal tissue in the ovule is not evident in $2x \times 4x$ and $4x \times 2x$ seeds of *Galeopsis* and cannot be an important cause of the seed abortion here. Endosperm formation always begins before the division of the eggcell; however, division of the eggcell sometimes fails, and developing ovules may in rare cases have endosperm but no embryo.

Development of endosperm and embryo was better when the tetraploid was maternal plant in the crosses. This conforms to a general rule in auto- and alloploid crosses, seed development being better when the maternal plant has the higher chromosome number. Exceptions to this rule are, however, known. An interesting reciprocal difference concerns the micropylar haustorium. In $2x \times 4x$ an extraovular outgrowth is formed as in normal diploid seeds: in $4x \times 2x$ an outgrowth was never formed. This shows that the structure of the somatic tissue of the ovules influences the behaviour of the endosperm. Tetraploid *G. pubescens,* more rarely than the diploid, forms such an outgrowth, which breaks through the wall near the bottom of the upper chamber, not near the top as in the diploid. When a breaking-through is initiated, although not completed, in $4x \times 2x$, it is near the bottom of the chamber as in $4x \times 4x$. It seems probable that the structure of the diploid ovule is more suitable to the formation of an opening in the wall than is the tetraploid ovule. The formation of an opening in $2x \times 4x$ seeds shows that the tetraploid endosperm is very vigorous at this early stage of development, a fact which makes the rapid degeneration soon afterwards seem strange.

Ovules with a slow and interrupted endosperm development occur occasionally in diploid, and often in tetraploid *G. pubescens.* Insufficient differentiation of the endosperm tissue was also observed here: the connection in the chalaza with the nutrients coming from the vascular bundle is weak; the elongated cells forming a link in this connection are small; there are no very large cells near the isthmus;

and the outermost layers of the endosperm form no cytoplasm-rich meristeme or absorbing tissue. Thus, no new structures are seen in $2x \times 4x$ and $4x \times 2x$ endosperm tissue; and that which seems to occur is a general starvation of the endosperm in all ovules. The faulty organization of the nutrient-absorbing parts of the endosperm is probably an indication of weak development of the endosperm rather than an initial cause of this weakness. On the other hand, these defects of the young endosperms must influence their further development negatively.

Changes in the somatic tissue of the ovule, especially of the endothelium surrounding the embryosac, have been considered the cause of the degeneration of the endosperm (COOPER and BRINK, 1945) or endosperm + embryo (SATINA, RAPPAPORT and BLAKESLEE, 1950). In *Datura* the endothelium cells, which are assumed to function as nurse cells to the endosperm, in normally developing seeds are flattened and destroyed by the growing endosperm about twelve days after fertilization. In most aborting ovules of $4x \times 2x$ *Datura* the endothelium cells enlarge, some of them beginning to divide through periclinal walls; through these divisions a »tumoral tissue» is formed, which resorbs the endosperm, and in most cases the embryo as well. It has been shown that extracts from tumours in $4x \times 2x$ seeds of *Datura inoxia* inhibit growth of excised embryos of *Datura Stramonium*, whereas injected into a capsule, they cause seed abortion (RAPPAPORT, SATINA, BLAKESLEE, 1950). The authors suspect tumours which form substances digesting endosperm and embryo to be of general occurrence in cases of seed abortion. In *Lycopersicon* the endothelium persists as a layer around the endosperm; it is ascribed the function of destroying the cells of the integument and transferring their content to the growing endosperm. After matings leading to aborting seeds the endothelium behaves in an irregular manner: it becomes meristematic, forming a tissue which fills the place formerly occupied by the endosperm.

In *Galeopsis* the endothelium cells are rich in cytoplasm but they are flattened and destroyed by the broadening parts of the endosperm already two days after pollination, as SCHNARF (1917) states »zur Annahme einer ernährungsphysiologischen, etwa enzymauslösenden Funktion bieten sie keinen Anhaltspunkt». The endothelium cells near the chalaza are more persistent, but show no abnormal behaviour in $2x \times 4x$ and $4x \times 2x$. The endothelium probably is already degenerating when the abnormal development in the seed sets in: this may

explain the more normal behaviour in *Galeopsis*. Nor does any other part of the integument form a tumour. The changes of the integument tissue observed in aborting ovules of *Galeopsis pubescens* seem to be secondary: they are not initiated before the first signs of a slower and interrupted development of the endosperm are visible. Thus, they do not indicate a somatoplastic sterility in the earlier, more orthodox meaning of the term.

The interrupted growth of the ovule is resumed after fertilization—during the first two days at a very moderate rate. After four days the ovules have considerably increased in size in all combinations. The initiation of rapid growth seems to coincide with the beginning of embryo formation, but it is uncertain if it is a result of auxin formation from the very young embryo. But no doubt the volume of the endosperm is important, for there is a correlation between endosperm and ovule size. In ovules growing with a low rapidity, cavity formation in the integument is belated or does not occur. After 6 days $4x \times 2x$ ovules are smaller than the good ovules of the tetraploid; cavities are formed later than in $4x \times 4x$. $2x \times 4x$ seeds are, of course, much smaller than $2x \times 2x$ seeds after 6 days. Clearly degenerating or small endosperms and embryos cannot produce adequate growth substances, so that the further development of the ovules stops. In different angiosperms cultivation of excised embryos from seeds with degenerating endosperms has succeeded, but has also shown that at early stages the embryos require for their nutrition, not only minerals and sugar, but also in some cases vitamins, amino acids, nucleic acid and unknown substances from cocoa milk and malt extract (*cf.* SKOOG, 1951). *Datura* embryos develop when they have attained the size of 0,5 mm.; with cocoa milk, already when they have the size of 0,1 mm.; with malt extract, 0,3 mm. maize embryos develop. It may perhaps be profitable to try the cultivation of $4x \times 2x$ embryos of *Galeopsis pubescens*.

The cause of the embryo abortion in $2x \times 4x$ and $4x \times 2x$ *Galeopsis pubescens* is no doubt the degeneration of the endosperm; the degeneration of the endosperm apparently occurs because it is starved. The initial development of the endosperm is vigorous. The embryosac has contained nutrients sufficient for the early development; when these are consumed the difficulties of nourishing the endosperm in cases with changed chromosome relations between the endosperm and the maternal tissue are very soon apparent in *Galeopsis pubescens*. The somewhat better seed development in

$4x \times 2x$ than in *Tetrahit* × *pubescens* where a foreign *speciosa* genome is also involved has a parallel in *Lycopersicon*. The $4x \times 2x$ mating of *L. pimpinellifolium* results in the abortion of nearly all ovules while in $4x$ *L. pimpinellifolium* × $2x$ *L. peruvianum* many germinable seeds are produced (COOPER and BRINK, 1945).

SUMMARY.

The high seed sterility of autotetraploid *Galeopsis pubescens* is caused by the absence of a normal embryosac in the ovule, failure of fertilization, or arrested development after fertilization. Peculiar traits in the seed development of the tetraploid are a more or less frequent failure of fusion of the polar nuclei, abnormally persistent antipodals, and a rather weak micropylar haustorium.

In $2x \times 4x$ crosses fertilizations are rare; the initial development of the endosperm is vigorous; but it is interrupted very early, and degeneration follows rapidly. In $4x \times 2x$ fertilizations are more frequent; endosperm development continues further but ultimately stops. The micropylar endosperm haustorium shows good development in the former cross, very weak development in the latter. The embryos are more long-lived than the endosperms. The cause of the complete seed abortion is probably due to changed chromosome relations between endosperm and maternal tissue.

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CHIASMA FORMATION AND CHIASMA INTERFERENCE IN THE ODONATA

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I. INTRODUCTION.

THE occurrence of negative correlation, *i. e.* positive interference, between cross-overs in neighbouring chromosome regions is one of the important discoveries of the early period of *Drosophila* genetics. When the genetical cross-over was later on identified with the cytological chiasma, the possibility of studying the mutual dependence of cross-overs in different segments of the same chromosome was essentially enhanced, even the genetically unexamined organisms being now brought within the reach of investigation. In the view of the cytological workers of the twenties and thirties an essential feature of chiasma interference was that positive interference takes place within one chromosome arm only, not being developed across the centromere. Later, however, some authors were able to show that this property, although present in most of the animals and plants studied, is by no means inherent in chiasma interference, but at least in certain cases the centromere is obviously transparent to interference, not forming any absolute barrier to it.

PÄTAU (1941) was the first to show this when studying the clear negative correlation between chiasma formation in different arms of the same chromosome in *Culex* and *Dicranomyia* (*Diptera Nematocera*). From this he drew the conclusion that positive interference extends across the centromere in both these species. Later CALLAN and MONTALENTI (1947), working with more abundant material, confirmed PÄTAU's result in regard to *Culex*.

In addition to these two species, positive interference across the centromere has been demonstrated in two further Dipterans, *Psychoda* sp. (MONTALENTI, 1946; CALLAN and MONTALENTI, 1947) and *Simulium equinum* (MONTALENTI, 1947); in the Orthopteran *Prionotropis appulum* (MONTALENTI and VITAGLIANO, 1947); in the earthworm *Eisenia foetida* (CALLAN and MONTALENTI, 1947); in the Crustacean *Asellus aquaticus* (MONTALENTI and VITAGLIANO, 1946); in the earwig *Forficula auricularia* (CALLAN, 1949); and in *Petunia violacea*, the only

plant species shown to possess the property in question (CALLAN and MONTALENTI, 1947).

Many years ago the present author (OKSALA, 1943) pointed out, in passing, that the same phenomenon has found strong expression in the male Odonata, being universal among them.

In most of these cases the existence of interference across the centromere has been concluded from the fact that a statistically proved negative correlation in chiasma formation exists between the two morphologically indistinguishable arms of a metacentric chromosome. This conclusion must be regarded as fully legitimate if we assume that both the arms of such a chromosome originally have an equal chance to form chiasmata, a presumption generally accepted by cytologists.

Since my study of the Odonata may furnish some additional data for the discussion of certain features of chiasma interference, it seems worth while to consider this problem in the light of the present observations in some detail.

The material basis for this consideration is furnished by a number of tetraploid spermatocytes showing multivalent formation. As an introduction it is necessary to describe the main features of the chromosome structure and chiasma formation in normal spermatocytes and oocytes of the Odonata, although a detailed account of them has been published earlier (OKSALA, 1943, 1945).

II. CHROMOSOME STRUCTURE AND CHIASMA FORMATION IN THE ODONATA.

1. CHROMOSOME STRUCTURE.

The chromosome complements of the different species of Odonata are on the whole uniform. The haploid chromosome numbers of some 40 European species which have been cytologically examined by the present author are 12, 13, and 14. Genera and even families are characterized by a certain number, deviations from this rule being rare exceptions. The male is always heterogametic, representing the XO-type.

Differences in size between the chromosomes of a complement are slight. Sometimes, however, the largest or the smallest chromosome, or both, can be distinguished from the others. In shape the chromosomes are also fairly uniform. All the species in which spermatogonial or somatic mitoses have been studied possess chromosomes of one type only. This is metacentric with arms of exactly the same length, if micro-

scopic evidence is to be relied upon. In species of the genus *Aeschna* this fact has been verified by a detailed analysis made earlier (OKSALA, 1943).

The centromere is situated in a clear median constriction. The chromosomes are also often typically bent at this point. The direct microscopic evidence thus argues in favour of a localized centromere in the Odonata. The postreductional type of meiosis characteristic of these insects has, however, caused certain authors (DE CASTRO, 1951; HUGHES-SCHRADER, 1948; LIMA-DE-FARIA, 1949; SCHRADER, 1949) to doubt the presence of a localized centromere in this group. Although the monocentric character of the chromosomes has not been experimentally proved so far, the empirical facts presented above are against its being diffuse. Another question is whether the localized centromere of the dragonflies is in all respects comparable with the centromere of, say, the Orthoptera or Diptera. These questions must, however, be passed over in this connection.

I have not been able to observe any heterochromatic segments in the chromosomes of the Odonata.

2. CHIASMA FORMATION.

A. DIPLOID SPERMATOCYTES.

Chiasmata in the normal (diploid) spermatocytes of the Odonata are formed according to one clear pattern (OKSALA, 1943). With very few exceptions every bivalent contains only one chiasma. Of the two morphologically, at least, similar arms only one forms a chiasma, the other remaining without one. At diplotene the bivalents reveal a cross-shape, first a »primary cross», later on a symmetrical cross lying in one plane. When diakinesis is reached the chiasma is, as a rule, more or less terminalized and appears as a typical constriction in the middle of a fairly compact rod bivalent. Since the meiotic mitoses occur postreductionally in the dragonflies (OKSALA, 1943, 1948), such bivalents are situated in the first division metaphase plate at right angles to the spindle. The terminal chiasmata are consequently seen as constrictions in the middle of the bivalent in polar views of the metaphase plates.

This is the rule. The exceptional formation of a chiasma in both the arm pairs of a bivalent is extremely rare. In my material I have found only a few ring bivalents, which are the result of such an occurrence. Statistics made on *Aeschna crenata* (OKSALA, 1943) show that 0,01 % of the bivalents are rings, the rest being rods. When search-

ing for ring bivalents several samples of a hundred cells have usually to be examined before a ring is found. Statistically they are thus negligible and can be discounted.

Univalents caused by a failure of chiasma formation are at least as rare as are bivalents containing two chiasmata. When attempting a causal analysis of this unique mode of chiasma formation, attention must naturally in the first place be directed to the pairing properties of the chromosomes in the earliest stages of meiosis. In the male dragonflies these stages are characterized by the following facts.

In the primary spermatocytes a clear and typical polarization appears. In leptotene the thin chromosome threads, which form a tangled mass, are pressed to one side of the nuclear membrane (»synizesis»). When pairing continues during zygotene, a process which cannot be directly followed, a typical bouquet is formed. The bouquet attains its final shape in pachytene. Now it can be seen clearly that the two ends of all the chromosomes are gathered at one point from which the chromosomes form arches which fill the whole nucleus. The number of these arches is one less than the haploid chromosome number, as the X-chromosome is situated as a heterochromatic body at the narrow end of the bouquet. The compact structure of the pachytene threads suggests that pairing is complete and intimate. This is further borne out by the fact that not even short unpaired regions can be discerned in them.

The pairing properties just described do not explain why chiasma formation occurs in one chromosome arm only. One possible explanation for this peculiar fact would be to assume a situation in which the pairing begins in one arm and is not able to continue across the centromere to the other. Since, however, both the chromosome arms are polarized and a typical bouquet is present this seems highly improbable. A much more probable explanation is that the two arms of each chromosome pair independently, and that initially each has the same chance to form a chiasma. A direct proof of the independence of the chromosome arms in pairing is, indeed, obtained from the multivalent formation in the tetraploid spermatocytes which will be considered in detail later.

Let us assume that not only pairing but chiasma formation, too, takes place in each arm completely independently of the possible chiasma formation in the other arm, *i. e.* that there is no correlation in this respect between the two arms. If chiasma statistics are available the expected frequency of ring bivalents, rod bivalents and pairs of uni-

valents can be calculated from them. In the following the method devised by CALLAN and MONTALENTI (1947, pp. 123—124) will be used.

Let the initial chance of every individual arm to form a chiasma be p. Its chance of not forming a chiasma is then $1—p$, or q. The probabilities of chiasma formation in both arms of a bivalent, in either or in neither, are accordingly p^2, $2pq$, and q^2, respectively. These values correspond at the same time to the probable formation of a ring bivalent, rod bivalent and a pair of univalents.

Since in the spermatocytes of the Odonata practically always only one chiasma per bivalent is formed, *i. e.* in 50 % of the arm pairs, the primary chance of each arm pair to form a chiasma $(p)=0{,}5$. On the above condition the expected percentage of ring bivalents, rod bivalents and univalent pairs is thus 25 %, 50 % and 25 %, respectively. The actual frequencies found deviate, however, strikingly from expectation. The fact that only rod bivalents are formed implies that a chiasma can be formed in a certain arm only if no chiasma is present in the other arm of the same chromosome. Hence, there prevails in this respect an almost absolute negative correlation between the two arms of one chromosome.

In my opinion *the only possible explanation for the formation of a single chiasma in these bivalents is a strong positive interference across the centromere*, an idea which I had advanced in 1943 (OKSALA, 1943, pp. 23 and 59).

For later comparisons we need to be able to determine the exact degree of this interference. Methods for the determination of a special *index of interference* have been developed by PÄTAU (1941) and by CALLAN and MONTALENTI (1947). In the following the latter method will be used.

CALLAN and MONTALENTI calculate two different values for p; p_1 is the chance of one chiasma to form in the one arm pair in the absence of a chiasma in the other; p_2 is the chance of chiasma formation in the one arm pair when a chiasma is present in the other arm pair of the bivalent. The values of p_1 and p_2 are obtained from the primary material. Thus, if the number of ring bivalents is a, the number of rod bivalents $2b$, and the number of univalent pairs is c, then $p_1=\frac{b}{b+c}$ and $p_2=\frac{a}{a+b}$. The index of interference is the complement of the ratio $\frac{p_2}{p_1}$, and it is calculated according to the formula $1-\frac{a(b+c)}{b(a+b)}$.

When the interference is absolute the value of the index is=1. In the absence of interference it is=0.

The index of interference for the spermatocytes in the Odonata is 1, which implies an absolute interference across the centromere. For the sake of comparison it may be mentioned that in *Culex* (CALLAN and MONTALENTI, 1947) this index varies in different individuals between 0,447 and 0,808.

B. DIPLOID OOCYTES.

Chiasma formation in the diploid oocytes is almost as invariable as in the spermatocytes, differing essentially, however, from the latter. Preparations representing diakinesis stages show that almost without exception only ring bivalents occur. Both arms of each chromosome form a chiasma. Each arm probably contains a single chiasma, although we do not possess complete proof of this point. Originally many of the chiasmata are interstitial, appearing terminalized, however, by the time that prometaphase is reached. Neither in the female nor in the male are the chiasmata localized. In the female also, the postreductional mode of division determines the situation of the bivalents in the first division metaphase plate. They are seen as rings in polar view, their centromeres being auto-orientated. Owing to a strong contraction the ring is often closed. An even more frequent situation is that the two chromosome components of a bivalent lie side by side in the metaphase plate, both the chiasmata having broken at this stage. A more detailed account of these phenomena is found in OKSALA (1945).

The primary pairing of the chromosomes is difficult to study in the oocytes. Observations on this process have consequently been made in a few species only. Of these *Libellula quadrimaculata* seems to have bouquet orientation, whereas in *Gomphus* species it is lacking.

The male and the female thus differ sharply in regard to chiasma formation. *In the female the strong interference across the centromere which is so characteristic of the male seems to be absolutely lacking.* The index of interference has not been calculated for the oocytes, as the material is too small for this purpose.

3. TETRAPLOID SPERMATOCYTES.

A. DESCRIPTION AND PRIMARY CONCLUSIONS.

The study of tetraploid spermatocytes occurring occasionally in normal testes has in many cases thrown fresh light on the factors which

govern chiasma formation and other meiotic processes. Several years ago I found a number of tetraploid spermatocytes in a testis of *Aeschna subarctica elisabethae* which have proved to be suitable material for the study of certain problems connected with chiasma formation in dragonflies.

This specimen of *Ae. subarctica elisabethae* was caught as an imago in Jyväskylä in Central Finland in the summer of 1936. Carnoy was used as fixative. This fluid, which in many cases does not come up to the standards of modern cytology, gives satisfactory results in the Odonata. For staining, Heidenhain's haematoxylin was used. The testis was sectioned at 15 μ.

The testis contained all stages of meiosis. The tetraploid spermatocytes, which represented prometaphase or metaphase stages of the first meiotic division, formed an island of about 60 (obviously 64) cells in a cyst which otherwise consisted of normal diploid spermatocytes at the same stage. The number of the tetraploid cells indicates that these cells must have been derived from one spermatogonial cell in which chromosome doubling had occurred six mitoses prior to meiosis. Since all the cells are bipolar without any extra centrioles it is more probable that the doubling resulted from endomitosis than from restitution.

Owing to the damage suffered by the cells on sectioning and the great number of chromosomes, it has been impossible to analyse all the cells completely. In normal diploid somatic cells of the male *Ae. s. elisabethae* 26 autosomes and one X-chromosome are found, which corresponds to 13 bivalents and one univalent X in the primary spermatocytes. In accordance with this each tetraploid spermatocyte ought to contain 52 autosomes, in different bivalent, multivalent and univalent combinations, together with 2 X-chromosomes. This exact number, *i. e.* 54 chromosomes in all, has been found in only 13 cells (in Table 1 Nos. 3, 4, 8, 12, 13, 24, 30, 36, 47, 48, 55, 56, 62). Nos. 1, 30, 31, 39 can, however, also be used in this analysis, as they lack only one X-chromosome, while all the autosomes which are important in this connection are present. In addition the configurations in eight further cells have been analysed completely; the total number of chromosomes in these, however, remain below expectation. Since, however, the deficit, which obviously depends on chromosomes having been lost at sectioning, is not great these cells have been included in the data presented in Table 1, which forms the basis of later discussion. The total frequency of multivalents, bivalents, and univalents in the material is seen in the bottom row of the table. When the X-chromosomes, which are strongly heteropycnotic throughout meiosis and thus naturally do not form any bi-

TABLE 1. *Complete analysis of 25 tetraploid spermatocytes in Aeschna subarctica elisabethae.*

IV=quadrivalents, III=trivalents, II=bivalents, I=autosome univalents, X=X-chromosome(s), A=autosomes, Chr.=chromosome, X:ta=chiasmata, Deficit=number of lacking chromosomes.

Cell No.	Ring IV	Chain IV	III	Ring II	Rod II	I	X	Chr. total	Deficit	X:ta	X:ta per autosome
1	2	2	—	1	17	—	1	53	1X, —	33	0,64
2	2	5	—	1	10	—	2	52	—, 2A	35	0,70
3	—	1	—	—	24	—	2	54	—, —	27	0,52
4	2	1	—	—	20	—	2	54	—, —	31	0,60
8	—	1	—	—	24	—	2	54	—, —	27	0,52
12	2	1	—	1	19	—	2	54	—, —	32	0,62
13	4	1	1	2	12	1	2	54	—, —	37	0,71
14	3	1	—	1	16	—	2	52	—, 2A	33	0,66
15	5	—	—	3	12	—	2	52	—, 2A	38	0,76
24	3	1	—	1	16	2	2	54	—, —	33	0,64
25	1	—	—	2	19	—	1	47	1X, 6A	27	0,59
30	—	2	—	—	22	—	2	54	—, —	28	0,54
31	—	2	—	—	22	—	1	53	1X, —	28	0,54
32	2	—	—	1	21	—	1	53	1X, —	31	0,60
33	3	—	—	3	15	—	2	50	—, 4A	33	0,69
36	4	—	—	1	17	—	2	54	—, —	35	0,67
37	2	3	1	1	9	1	2	46	—, 8A	30	0,68
38	1	2	—	3	16	—	1	51	1X, 2A	32	0,64
39	2	1	—	—	20	—	1	53	1X, —	31	0,60
47	1	1	—	1	21	—	2	54	—, —	30	0,58
48	3	1	—	—	18	—	2	54	—, —	33	0,64
54	1	1	—	3	18	—	2	52	—, 2A	31	0,62
55	2	1	—	3	17	—	2	54	—, —	34	0,65
56	—	3	—	3	17	—	2	54	—, —	32	0,62
62	3	5	—	—	10	—	2	54	—, —	37	0,71
Total	48	36	2	31	432	4	44	1316	6X, 28A	798	0,63

valents, are excluded the present material contains 318 sets of 4 *inter se* identical chromosomes.

The fact that the material contains some cells in which chromosomes are lacking entails the following error: The absence of a certain bivalent in the nucleus when its homologue is present, a situation of the highest probability, means that in the material this chromosome type is represented in one set by two homologues only. The presence of an odd bivalent in the material implies automatically the absence of a homologous bivalent (or alternatively that of two univalents). Therefore

the frequency of bivalents as compared with that of multivalents is somewhat smaller in our statistics than in reality. This fact is not taken into account in the following consideration, since the error caused by it must in any case be negligible, the whole deficit being only 28 autosomes.

These 318 sets of four chromosomes give rise to different configurations, as shown by Table 2.

TABLE 2. *Frequencies of different configurations found in the tetraploid spermatocytes of Aeschna subarctica elisabethae.*

Ring quadrivalent	48 cases	= 15,1 %
Chain quadrivalent	36 »	= 11,3 %
Trivalent-univalent	2 »	= 0,6 %
Two bivalents	231 »	= 72,7 %
One bivalent and two univalents	1 case	= 0,3 %
Total:	318 cases	= 100,0 %

The complete chromosome sets of some of these tetraploid nuclei are seen in Figs. 3—15.

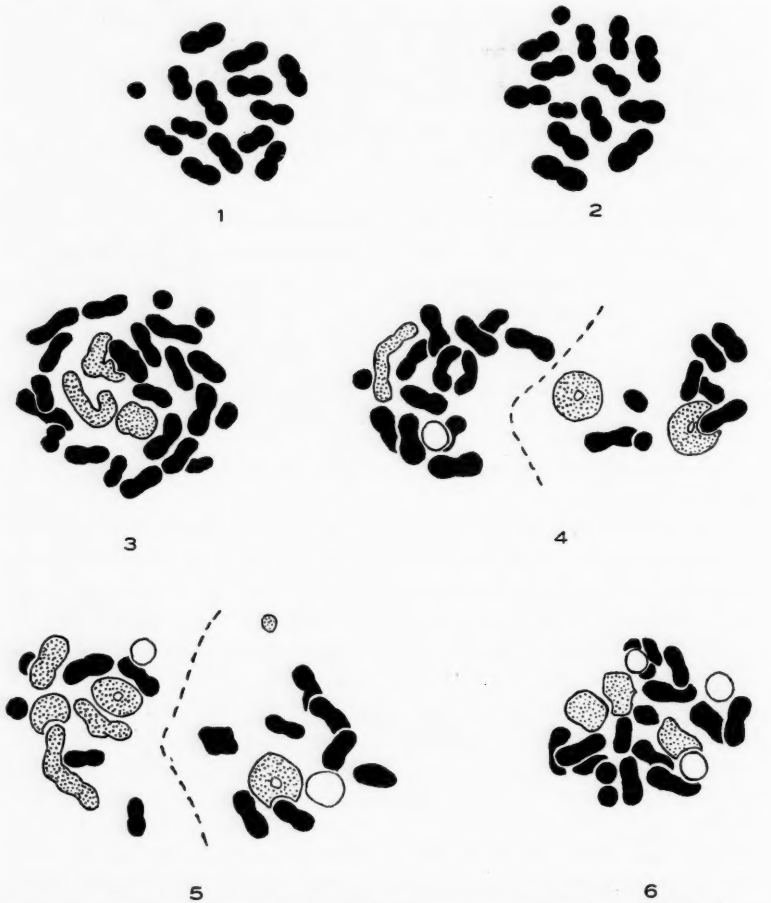
At full metaphase bivalents as well as multivalents are autoorientated in the plate, as can be expected in organisms with postreduction. Quadrivalents seem to be somewhat slower in their orientation than bivalents, as shown by the fact that sometimes a big ring quadrivalent is seen from the side in polar views of metaphase plates, although the bivalents are regularly orientated.

A special feature of the tetraploid spermatocytes is the occurrence of ring bivalents. Their number is, however, small, 31 in all, which means 1,24 rings per cell, or 6,7 % of the total number of bivalents. The difference is, however, significant as compared with the diploid spermatocytes, as is seen from the above account. In order to ascertain this fact I have examined 59 diploid metaphase plates in the same cyst to which the tetraploid spermatocytes belong, and I have been able to find only two configurations which could be interpreted as ring bivalents. In other words, in diploid spermatocytes we have only 0,07 rings per two cells or 0,26 % of the total number of bivalents.

The following conclusions suggest themselves immediately from the above facts and from the statistics in Tables 1 and 2.

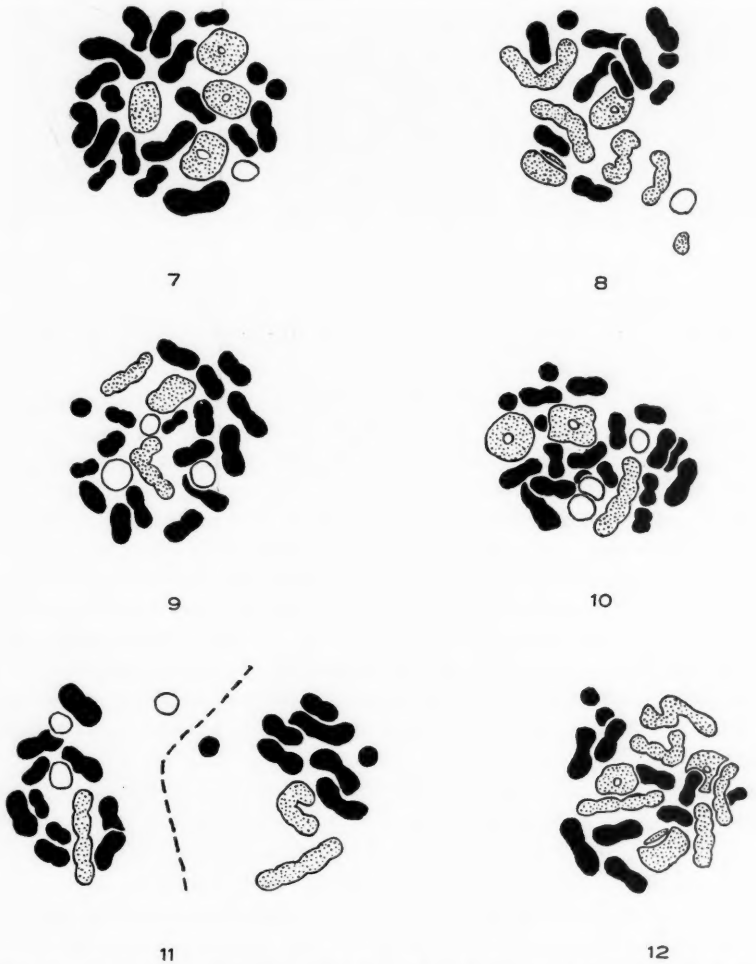
(1) The mean chiasma frequency, which in the diploid cells with practically no two-chiasmata bivalents is 0,50 per autosome, in the tetraploid cells is clearly higher, *i. e.* 798/1272=0,63 per autosome.

This deviates in a striking and interesting way from the known fact that the relative chiasma frequency in autotetraploids, as a rule, is



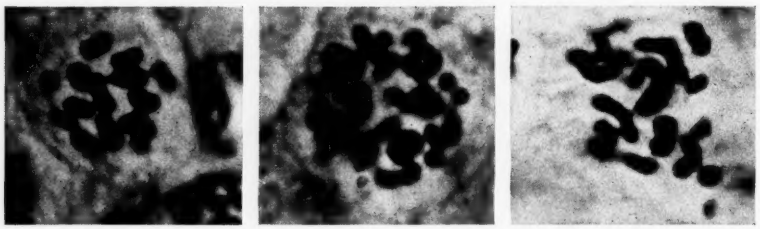
Figs. 1—6. *Aeschna subarctica elisabethae.* Prometaphase and metaphase I. — Figs. 1—2, normal diploid spermatocytes. — Figs. 3—6, tetraploid spermatocytes. Rod bivalents and X-chromosomes black, ring bivalents outlined, multivalents and autosomal univalents outlined and stippled. — Fig. 3, cell No. 4; Fig. 4, cell No. 12; Fig. 5, cell No. 13; Fig. 6, cell No. 33. Detailed explanations in Table 1. — Magnification 3000×.

lower than in the corresponding diploids. A corresponding unusual situation has been found by CALLAN in the tetraploid spermatocytes of *Forficula* (CALLAN, 1949). According to him, the mean chiasma frequency per nucleus in the diploid cells is 11,1, while in the tetraploid



Figs. 7—12. *Aeschna subarctica elisabethae.* Prometaphase and metaphase I. Tetraploid spermatocytes. — Fig. 7, cell No. 36; Fig. 8, cell No. 37; Fig. 9, cell No. 38; Fig. 10, cell No. 55; Fig. 11, cell No. 56; Fig. 12, cell No. 62. — Other explanations as for Figs. 3—6.

cells it is 24,71. Calculated per chromosome these values are 0,51 and 0,56, the difference between these two figures being somewhat less striking than in *Ae. s. elisabethae.*



13 14 15

Figs. 13—15. *Aeschna subarctica elisabethae.* Microphotographs of metaphase I. — Fig. 13, a diploid spermatocyte; Figs. 14—15, tetraploid spermatocytes. Fig. 13, the same cell as in Fig. 1; Fig. 14, cell No. 36 (*cf.* Fig. 7); Fig. 15, cell No. 37 (*cf.* Fig. 8).

(2) In the tetraploid cells 86 (=27 %) of the 318 possible multivalents are realized. Since the formation of a multivalent requires the pairing of the two arms of one chromosome with different partners, it is clear that in *Ae. s. elisabethae* the two arms of one chromosome are able to pair independently in zygotene. This is readily understandable if we remember the metacentric structure of the chromosomes and their clear polarization and bouquet formation in zygo-pachytene. The maximal number of 8 multivalents in one cell shows that at least 8 of the 13 autosomes are able to behave in this manner. Since, as is seen from the figures, multivalents are formed by the small as well as by the large members of the chromosome complement it is obvious that in all the chromosomes the two arms are at least potentially independent in their pairing.

(3) All the multivalents observed are simple rings or chains; no more complicated configurations, for instance branched multivalents, are discernible. Thus, an individual chromosome arm seems never to form a chiasma with two different partner chromosomes.

(4) The frequency of ring bivalents is significantly higher than in the diploid cells.

(5) The occurrence of multivalents and ring bivalents shows that in a number of cases a chromosome is able to form a chiasma in both its arms, a situation practically never appearing in diploid cells. *The almost absolute chiasma interference across the centromere characteristic of the latter is, thus, significantly weakened in the tetraploid cells.* Exactly the same situation has been found by CALLAN in *Forficula.*

In order to determine this weakening exactly, the index of inter-

ference can be calculated. The same formula as before can be used for these tetraploid cells. In this case it must be remembered that in the primary data a ring quadrivalent corresponds to two ring bivalents, a chain quadrivalent corresponds to one ring and one rod bivalent, and a trivalent-univalent to one ring bivalent and one pair of univalents. We then obtain the value 0,4188 for the index of interference in the tetraploid spermatocytes.

B. INTERPRETATION OF THE CHROMOSOME BEHAVIOUR.

It has been shown above that chiasma interference is weaker in the tetraploid spermatocytes now under consideration than in normal diploid ones. This can be seen from the fact that interference in some cases does not act across the centromere from one arm of the chromosome to the other, thus allowing for the formation of multivalents on the one hand and ring bivalents on the other hand.

We must now attempt to discover the cause of this phenomenon.

In order to do so, we must first study the rules governing the occurrence of chiasmata in the tetraploid nuclei. For this purpose we must try to find out what conclusions can be drawn from the structure and relative number of the different configurations in the present material.

To obtain a necessary theoretical background for this discussion we must first analyse the general conditions for the formation of multivalents.

a. The theory of multivalent formation.

For a multivalent to be formed from three or more homologous chromosomes the following conditions must be fulfilled:

(1) At least one of the chromosomes must exchange partner when the chromosomes pair in zygo-pachytene. When this happens a *primary or pachytene multivalent* is formed. If every chromosome pairs with one partner only, multivalent formation is already made impossible at this stage.

(2) In diplotene the requisite number of chiasmata must arise to prevent the primary multivalent from falling apart into bivalents or univalents. At least two chiasmata are needed, one on each side of the point of exchange of partner. In this way a *definite multivalent* is obtained which lasts throughout the whole meiotic prophase and into the meiotic mitoses.

What kind of multivalent will arise depends not only on the number of homologous chromosomes taking part in it, but also on the number and situation of the points of exchange of partner, and those of the chiasmata.

These general conditions for the formation of multivalents have been the subject of a detailed study by KLINGSTEDT (1937). The main point under discussion is the pairing block concept, which has been introduced into cytology by DARLINGTON and MATHER (1932) and by STONE and MATHER (1932). In the words of KLINGSTEDT (1937, p. 201): »Thus a pairing block is to be defined as *a region of a bivalent thread, the pairing of which has arisen as a consequence of one initial point of pairing.* The number of pairing blocks is equal to the number of independent initial points of pairing».

If the pairing of a chromosome starts from one initial point, *i. e.* it has only one pairing block, no exchange of partner is possible. Consequently, such a chromosome type cannot form multivalents however great a number of homologues may be present, and however many chiasmata may be formed at later stages. KLINGSTEDT further shows that if in a triploid or tetraploid nucleus the homologous chromosomes pair with two pairing blocks the probability of their forming multivalents is $^2/_3$ (66,7 %) and of their producing two bivalents (in a tetraploid), or a bivalent and a univalent (in a triploid), only $^1/_3$ (33,3 %). In the tetraploid this is due to the fact that in each of the four identical chromosomes there is twice the probability of its two pairing blocks pairing with a different partner than with the same partner. In the triploid again, in which only three homologues are present, the corresponding situation depends on the fact that in each chromosome there is twice the probability of its pairing with only one of its pairing blocks than with both or with none of them. The probability of multivalent formation steadily increases with the increase in the number of pairing blocks, as $n+1$ pairing blocks always give more multivalents than n, the difference being $^2/_3$ of that fraction which in the case of n pairing blocks did not give any multivalents.

Thus, theoretically, the number of pairing blocks determines absolutely what possibility each chromosome type has to form multivalents. The pairing block concept is, thus, of the greatest importance for the theory of multivalent formation. The analysis of this concept made by KLINGSTEDT is in my opinion most thorough, and consequently the pairing block theory will not be considered further in this connection. It must, however, be stressed that a pairing block must not be

thought of as a fixed region in the chromosome. It is not in the first place a morphological but a functional concept. Depending on the great number of factors influencing chromosome pairing the number and length of pairing blocks varies even as regards one chromosome. Only in the cases in which pairing, owing to some special mechanism, *e. g.* strict polarization, is very regular, would it be possible to regard a pairing block in a statistical sense as a relatively constant morphological unit.

The conditions determining the formation of multivalents are, thus, theoretically obvious and easy to define. In each actual case it is, however, difficult to predict what kind of multivalents will be formed, and in what proportions. This difficulty naturally arises from the fact that the number of pairing blocks and their lengths cannot be determined directly. Conclusions concerning them can be drawn only from multivalent statistics. In addition a number of other factors affecting chromosome pairing and chiasma formation play a part in the formation of multivalents.

In those cases, however, in which the chromosome complement is very homogeneous, in that the individual chromosomes are similar in size and structure, and the number of pairing blocks can be predicted with fair accuracy, we can theoretically calculate the frequencies of the different kinds of expected configurations.

The simplest case of autotetraploids, to which the present discussion is solely restricted, provides a situation in which the chromosomes always pair with two equal pairing blocks. Let us now consider theoretically the formation of multivalents in such a case, assuming that each pairing block has initially the same chance to pair and to form one chiasma with its homologue. It is easiest to assume that all the chromosomes are exactly metacentric and that each arm corresponds to one pairing block.

On this assumption two bivalents are the result in $^1/_3$ of all the cases, as the probability of an exchange of partner is only $^2/_3$ when we have two pairing blocks.

In the latter fraction of $^2/_3$ of cases the exchange of partner is realized in pairing and if enough chiasmata form, the result is a definite multivalent. The probability of each possible configuration in this fraction of cases is easy to calculate, if we know the probability with which an arm pair definitely pairs, *i. e.* through chiasma formation. This figure, again, is obtained simply by dividing the number of paired arm pairs in the material by the total number of arm pairs. Naturally

the justifiable assumption must again be made that pairing and non-pairing take place completely at random.

Let this figure, *i. e.* the probability with which each arm pair pairs, be, as above, $=p$. The probability with which each arm pair does not pair is then $1 - p = q$. The probabilities with which two arbitrary arm pairs pair or do not pair simultaneously are then p^2 and q^2, respectively, those of three arm pairs p^3 and q^3, and those of four arm pairs p^4 and q^4, respectively.

The probability of the simultaneous pairing of the four arm pairs

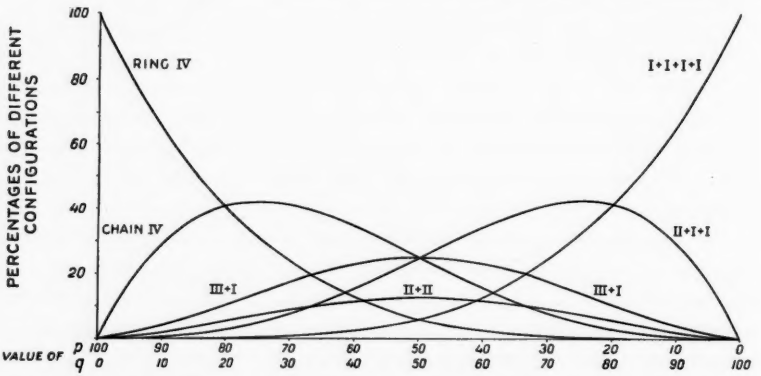


Fig. 16. Graph showing the probabilities of different configurations for any value of p in an autotetraploid with metacentric chromosomes, each having two pairing blocks and one exchange of partner in pairing. — Further explanations in the text.

is naturally at the same time the probability of the formation of a ring quadrivalent. And correspondingly the probability of the simultaneous non-pairing of four arm pairs is the probability of all the four homologues remaining as univalents.

The formation of a chain quadrivalent requires the pairing of three arm pairs and non-pairing of one arm pair simultaneously. The probability of this situation is naturally p^3q. In regard to a single configuration, however, this can be realized in four different ways, since any of its four arm pairs (A, B, C, or D; see Fig. 17) may remain unpaired while the three others pair. The actual probability with which a chain quadrivalent forms is thus $4\,p^3q$.

The probability of two arm pairs pairing in one configuration while the two others remain unpaired is p^2q^2. In regard to one configuration this situation can be realized in six different ways, of which

four (the paired arm pairs *inter se* and the non-paired ones *inter se* being non-homologous: in Fig. 17 A and C, A and D, B and C, B and D) give a trivalent-univalent, and the two others (paired arm pairs *inter se* and non-paired ones *inter se* being homologous: in Fig. 17 A and B, C and D) give two bivalents. The probability of a trivalent-univalent's occurring is thus $4\,p^2q^2$ and that of two bivalents $2\,p^2q^2$.

There is one possibility left, the falling apart of the configuration into a bivalent and two univalents. A condition precedent is the pairing of one arm pair while the others remain unpaired. Since this situation, too, can be realized in regard to any configuration in four ways, its probability is $4\,pq^3$.

According to these formulae it is possible to calculate the prob-

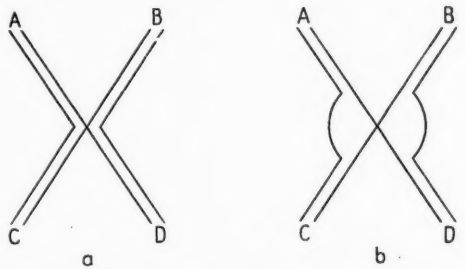


Fig. 17. Zygotene pairing of four homologues with one exchange of partners. Pairing is complete in *a*, incomplete with an unpaired centric region in every chromosome in *b*. The four arm pairs marked A, B, C, and D.

ability of each configuration for every possible value of p. To make this clear Table 3 contains the values of p and q and the calculated probabilities for the configurations mentioned above at values of p at every interval of 10 % between 100—0. From these values a graph (Fig. 16) has been computed which shows the probability of each possible configuration for any value of p.

This graph reveals certain interesting rules of multivalent formation. In this connection we will consider the trivalent curve only, which lies under both the quadrivalent curves as long as the probability with which the arms pair is considerable. This explains simply and clearly the often stated fact (*e. g.*, DARLINGTON, 1937, p. 126; WHITE, 1934) that in autotetraploids, in which the chromosomes, as a rule, pair with at least two about equally effective pairing blocks and consequently form a considerable number of multivalents, the frequency of trivalent-

TABLE 3. *Probability of different configurations being formed with different values of p and q in an autotetraploid with metacentric chromosomes in the case of one exchange of partners in pairing.*

IV=quadrivalent, III=trivalent, II=bivalent, I=univalent. Further explanations in the text.

p	q	Ring IV p^4	Chain IV $4p^3q$	III + I $4p^2q^2$	II + II $2p^2q^2$	II + I + I $4pq^3$	I + I + I + I q^4	Total
100,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	100,00
90,00	10,00	65,61	29,16	3,24	1,62	0,36	0,01	100,00
80,00	20,00	40,96	40,96	10,24	5,12	2,56	0,16	100,00
70,00	30,00	24,01	41,16	17,64	8,82	7,56	0,81	100,00
60,00	40,00	12,96	34,56	23,04	11,52	15,36	2,56	100,00
50,00	50,00	6,25	25,00	25,00	12,50	25,00	6,25	100,00
40,00	60,00	2,56	15,36	23,04	11,52	34,56	12,96	100,00
30,00	70,00	0,81	7,56	17,64	8,82	41,16	24,01	100,00
20,00	80,00	0,16	2,56	10,24	5,12	40,96	40,96	100,00
10,00	90,00	0,01	0,36	3,24	1,62	29,16	65,61	100,00
0,00	100,00	0,00	0,00	0,00	0,00	0,00	100,00	100,00

univalents is very small as compared with that of the quadrivalents and bivalent pairs. Calculations, which, however, cannot be presented in this connection, show that the percentage of trivalents in the multivalent fraction is considerable only if the probability with which the two pairing blocks pair is strikingly dissimilar, and the probability of even the »longer» pairing block is fairly small. In this case the total percentage of the multivalents is naturally very low.

b. Expectation of chiasma formation and its comparison with empirical data.

The above detailed ratiocination has been presented because the *Aeschna* case now under discussion fulfils rather well the conditions stated earlier. The chromosomes are fairly equal in size. The size differences present are certainly so small that they do not decisively affect the cell mechanics of the chromosomes. Since in addition all the chromosomes are metacentric, all the chromosome arms have the same chance to pair. This is further facilitated by the regular bouquet orientation, as a result of which all the chromosome ends are near each other when pairing begins. The choice of partner must consequently occur quite at random. Moreover, it must not be forgotten that the independent pairing of the chromosome arms is not only a theoretical

possibility but an ascertained fact which is proved by the formation of multivalents. This holds true in regard to the large as well as the small members of the chromosome complement. The chromosomes thus pair with two pairing blocks, and these blocks correspond statistically, at least roughly, to the chromosome arms.

Theoretically, it is naturally possible for individual chromosomes to pair with more than two pairing blocks. This would entail an exchange of partner occurring twice or more in one chromosome. This would give rise to certain irregularities in multivalent formation, *e. g.* branched multivalents or a considerable fraction of univalents. Since, however, all the observed multivalents are regular rings or chains and the frequency of univalents is negligible, the simultaneous existence of more than two pairing blocks occurs quite exceptionally, if at all. Moreover, the occurrence of more than two pairing blocks is not important in this connection, the essential point being that the number of pairing blocks is at least two.

Let us consider the present *Aeschna* case against this theoretical background.

Our presumption that the arms act as independent pairing blocks means that in $^2/_3$rds of the cases an exchange of partner ought to take place amongst all the four homologues, *i. e.* multivalents ought to arise in this proportion of cases. The expected frequency of multivalents is thus $^2/_3 \times 318 = 212$. In the material we find only 86 multivalents, the deficit (126) thus being considerable. The same figure naturally appears as an excess in the number of pairs of bivalents; the expectation is 106 pairs of bivalents, but the material contains 232 of them (including one pair of univalents).

It is thus obvious that the above theory is not in accord with the data in our case. To this question we shall return later. We must now consider in detail chiasma formation in the tetraploid spermatocytes. This is done separately in regard to the multivalent and the bivalent fractions.

The first step is to analyse *how chiasma formation is determined in the multivalents.* If we assume that chiasma formation in the primary multivalents, *i. e.* in cases when the exchange of partner in pairing is realized, takes place completely at random in each arm pair, we can first calculate, from the chiasma statistics, the primary chance of an individual arm pair to form a chiasma, or in other words to pair definitely. Secondly, it is possible to calculate the probability of formation of each possible configuration by the method theoretically deduced above.

Since the material contains 86 multivalents, we have in this fraction $4 \times 86 = 344$ arm pairs which must be regarded as having the same

TABLE 4. *Actual and expected frequencies of different configurations in the multivalent fraction in the tetraploid spermatocytes of Aeschna subarctica elisabethae.*

	Ring IV		Chain IV		III+I		II+II		II+I+I		I+I+I+I		Total	
	No	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Found	48	55,8	36	41,9	2	2,3	—	—	—	—	—	—	86	100,0
Expected	52,3	60,8	27,7	32,2	3,7	4,3	1,8	2,1	0,5	0,6	0,0	0,0	86	100,0

primary probability of forming a chiasma. According to the chiasma statistics, 304 of these have actually formed a chiasma. The primary chance to form a chiasma (p) is thus $304/344 = 0{,}883$. The theoretical expectation of the relative frequencies of the different configurations in this actual case is given in Table 4 both as the percentages and as the absolute figures. The empirical data are also recorded similarly as percentages and absolute figures. It is seen that, despite the small material, the values found agree fairly well with those calculated. According to the theory, a very small number of bivalent pairs ought to arise even when there is an exchange of partner. Their frequency should be one half of the frequency of the trivalent-univalents. Since the material contains two trivalents, one pair of bivalents ought to be present also. This again would somewhat decrease the primary chances of chiasmata forming, which in turn would make the distribution of the configurations even more ideal (see Fig. 16).

The above considerations show that *chiasma formation in the multivalents is completely at random.* No correlation in regard to chiasma formation is found between the two arms of one chromosome. The conclusion thus seems inevitable that *in the definite multivalents chiasma interference has not acted across the centromere from one arm to the other.* An even more exact way of expressing this fact is to calculate the index of interference for the multivalent fraction. If we keep in mind what has been said above concerning the application of the formula of CALLAN and MONTALENTI to multivalents, the value of the index is 0,02, which means a practically total lack of interference.

The situation is completely different with regard to the bivalent fraction of the spermatocytes. Although the frequency of ring bivalents is noticeably higher than in the diploid spermatocytes, the frequency of rod bivalents is so disproportionately high that *there must be a clear interference across the centromere in bivalents.* The same can be con-

TABLE 5. *Actual and expected frequencies of ring bivalents, rod bivalents and univalent pairs in the bivalent fraction in the tetraploid spermatocytes of Aeschna subarctica elisabethae.*

	Ring II		Rod II		I+I		Total	
	No.	%	No.	%	No.	%	No.	%
Found	31	6,7	432	93,1	1	0,2	464	100,0
Expected	131,3	28,3	231,1	49,8	101,6	21,9	464	100,0

cluded from the fact that, as shown earlier, the index of interference is relatively high in the tetraploid spermatocytes although, as regards their multivalent fraction, it is almost zero.

The 31 rings, 432 rods, and 1 univalent pair making up the bivalent fraction of the material contain 928 arm pairs in all. Of these only 494 have formed a chiasma. If the chiasmata were formed at random the primary chance of every arm pair to form a chiasma (p) would be $494/928 = 0{,}532$. The frequencies of ring bivalents, rod bivalents, and pairs of univalents expected on this basis, together with those found, are presented in Table 5 both as percentages and as absolute figures. The calculated and empirical values are in striking disagreement. The index of interference in this case is 0,874, which shows a strong interference across the centromere, which, however, is not absolute as in the diploid spermatocytes.

c. Reason for break-down of chiasma interference in multivalents.

As interference across the centromere is totally lacking in multivalents, whereas it is present in bivalents, the interpretation immediately suggests itself that the exchange of partner is the factor which interrupts the interference. This conclusion has been reached by CALLAN in his paper on the tetraploid spermatocytes of *Forficula*. As CALLAN puts it (1949, p. 212): »The present study proves that this interference is removed or at least depressed if in chromosome pairing an exchange of partner takes place. Chiasma interference must thus be considered as a relational property shared by two partner chromosomes and not transmissible along the length of one of the chromosome threads beyond the point where the pairing relationship has been altered».

In my opinion it is not so obvious that the factor which breaks

down the interference in multivalents is the exchange of partner in itself, as postulated by CALLAN. Some other factor which is absent in the diploid nuclei but present in the tetraploid ones, might equally well be the cause. It must not be forgotten that in the case of *Aeschna* the interference is somewhat weakened even in the bivalents in the tetraploid spermatocytes, as proved by the occurrence of ring bivalents; and in these the phenomenon cannot, of course, be caused by an exchange of partner. (In *Forficula*, studied by CALLAN, ring bivalents have not been found.)

Before we make a final attempt to analyse the factors determining chiasma formation in the tetraploid nucleus, we must try to ascertain the reason for the small number of multivalents and the strikingly large number of bivalents in our material, a question passed over in the earlier discussion.

The excess of bivalents in this case can be explained theoretically in four different ways:

(1) In the tetraploid nucleus the great number of chromosomes hinders normal pairing, and the result is that the numerous chromosome arms do not find their partners in the course of the period suitable for pairing (»time limit to pairing»).

(2) The greater distances in the tetraploid nucleus cause the following situation which gives rise to bivalents: When two chromosome arms have once paired, the other two arms have a greater probability to come into contact with each other than with other homologous chromosomes.

(3) Pairing takes place so quickly that when two chromosomes have initiated their pairing in the distal ends of one arm pair the pairing continues to the other arm pair without allowing for a free choice of partner in the latter. In other words, although according to our original presumption each chromosome potentially possesses two pairing blocks, in practice they would often pair with one pairing block, thus forming more than the expected number of bivalents.

(4) Primary multivalents fall apart into two bivalents at diplotene owing to a failure of chiasma formation.

Let us consider each of these possibilities separately. As regards the first, it is obvious that the chromosomes may hamper each other and so prevent the homologous chromosome arms from coming into effective contact. This disturbance ought, however, to affect the two non-homologous arm pairs of the primary multivalent, and thus lead to the formation of a trivalent-univalent, twice as often as the two

homologous arm pairs with resulting bivalent formation. Since, however, the material contains only two trivalents the mutual disturbance of the chromosomes must be a negligible factor in the production of extra bivalents.

The second of the above-mentioned possibilities is equally impossible as a cause of the excess of bivalents in the tetraploid cells. Firstly, the nuclei in the tetraploid spermatocytes are not large enough for even the maximal distances in them to exert a decisive influence on the pairing mechanics of structures as long as the zygo-pachytene chromosomes in *Aeschna* species. And bearing in mind that we have a typical polarized pairing with a bouquet stage, the distance factor cannot play any rôle in chromosome pairing in the tetraploid nuclei.

There thus remain the third and the fourth possibilities. The choice between these two alternatives is not easy. A special difficulty is that we do not know how rapid a process chromosome pairing is in the Odonata. The completeness of the pairing observed in the pachytene stages of dragonflies seems to argue in favour of its being rapid. On the other hand it might equally well be an indication that the time limit to pairing is not very strict. In any case it seems highly improbable to me that the pairing could be so rapid that when it has begun in the chromosome end which first finds a partner, it would continue across the centromere to the distal end of the other arm before the latter had time to find a partner. Such an event is especially improbable in the present case where, owing to polarization, the chromosome ends are already near each other when the pairing begins. And even supposing that pairing could sometimes occur in this way, it can scarcely be thought that this would happen in the majority of cases.

A comparison with other tetraploid spermatocytes described in the literature corroborates this idea. It is true that organisms which would be directly comparable with *Aeschna* are rare. Certain Orthoptera, however, in which the chromosomes contain two or more pairing blocks and in which both chromosome ends are polarized, offer a suitable parallel. Thus the long, nearly acrocentric, chromosomes of *Schistocerca* (WHITE, 1934) form 62,5 % quadrivalents in the tetraploid spermatocytes. The meiosis of *Callimantis antillarum* described by HUGHES-SCHRADER (1943) corresponds even better to that of the Odonata. The majority of the chromosomes are metacentric and they obviously pair with two blocks, both chromosome ends being polarized. The percentage of quadrivalents is 51,8 %. This must, however, be regarded as the minimum number, since, as especially pointed out by

HUGHES-SCHRADER, the cells containing the maximal number of quadrivalents have been left out owing to the difficulty of analysing them. KLINGSTEDT (1937), again, in *Chrysochraon dispar*, has found that the long chromosomes with a submedian centromere form 37,5 % quadrivalents. He points out, however, that here we obviously have a localized pairing which decreases the chiasma frequency and at the same time the probability of quadrivalent formation. In accordance with this, fairly high multivalent frequencies are found in the meiosis of many plants in which there is no polarization.

In all these cases pairing must have begun independently at several points. In view of these examples pairing cannot, at least as a rule, be regarded as a very rapid, almost instantaneous process.

Taking all this into consideration the percentage of multivalents, *i. e.* 27 %, in *Ae. s. elisabethae* is surprisingly low, especially as the conditions for multivalent formation ought to be ideal. Multivalent frequencies as low or lower than this among metacentric or fairly metacentric chromosomes have been reported only in *Forficula* (CALLAN, 1949) and in *Culex* (MOFFETT, 1936). In the former the percentage of multivalents is 31,8, in the latter as low as 8,3, the material in this case consisting, however, of 8 cells only. It must not be forgotten that these two are organisms in which interference has been proved to occur across the centromere and which, thus, belong to the same category as *Aeschna*. Again, in those cases, *e. g. Euschistus variolarius*, in which the tetraploid spermatocytes are completely devoid of multivalents, the chromosomes obviously pair with one block only.

In passing, this question may also be considered from the point of view of the selection theory. It seems highly improbable that both the chromosome ends would be polarized throughout the whole group of dragonflies if the pairing did not actually start from both ends. The optimal *index of recombination* is obviously achieved in the Odonata when the female forms two chiasmata per bivalent, and the male one. The initiation of the pairing at both the chromosome ends in the male obviously only secures the occurrence of this one chiasma, necessary for bivalent formation. The chiasma interference acting in normal spermatocytes again prevents the formation of more than one chiasma.

The fourth of the above possibilities, *i. e. failure of chiasma formation in the primary multivalents*, thus remains as the *most probable reason for the excess of bivalents* in the tetraploid spermatocytes of *Ae. s. elisabethae*. We can thus make the following calculation: The frequency of pachytene multivalents has originally conformed with expectation. In other words, it has been about $^2/_3$ of the total contingency of cases, *i. e.* $^2/_3 \times 318 = 212$. Since, however, an insufficient number of

chiasmata has arisen, the majority, 212 — 86 = 126, of the primary multivalents has fallen apart giving rise to bivalents. Of course, this theoretical calculation may deviate from the actual situation in that the number of primary multivalents may have been lower than expectation. This depends on the possibility that in addition to this chief factor decreasing the number of multivalents the third of the factors mentioned above has also played a rôle. Even in view of this it seems to be most probable that a considerable fraction, probably one half, of all the primary multivalents finally gives rise to bivalent pairs owing to lack of chiasmata.

If we accept this view, the next question is the reason for the falling apart of the majority of the primary multivalents.

Since when breaking up, the primary multivalents practically always produce bivalents, and not trivalent-univalents, it is evident that *chiasma formation in them is not a random process, but a strictly selective one.* Chiasmata in them are always situated in two *inter se* homologous arm pairs, while the two other *inter se* homologous pairs remain without. This again implies that in all the cases in which the primary multivalent falls apart there is a strong negative correlation in regard to chiasma formation between the two arms of one chromosome. In other words, *in a certain fraction of the primary multivalents chiasma interference acts, as a rule, across the centromere, and even across the point where the partners are exchanged.*

Why, however, in certain other cases, does interference break down, allowing the formation of definite multivalents with purely random chiasma formation?

The reason must obviously be the same as that which in the primary bivalents sometimes interrupts the interference and thus gives rise to ring bivalents.

A natural explanation for both these cases is provided by a factor which is always and inevitably present in polyploid meiotic nuclei. It is known that pairing is somewhat hindered and delayed in polyploid cells as compared with diploid ones. The relatively lower chiasma frequency in allo- and autotetraploids than in the corresponding diploids has been explained on this basis (*e. g.*, UPCOTT, 1939).

In my opinion pairing in these cells is hindered and retarded, so that when it begins from the distal ends of the chromosomes it has not time to become complete in all cases. Accordingly, *there remains in the middle of the chromosomes, probably most often on both sides of the centromeres, i. e.* in the proximal region of the chromosome arms, *an*

unpaired region which interrupts the interference (Fig. 17). If pairing in this case has begun without an exchange of partner and no other factor prevents the formation of a chiasma in both the arms the result is a ring bivalent. If, again, we have a case in which pairing has begun with an exchange of partner a multivalent is formed. The purely random chiasma formation in turn determines the character of the resulting multivalent.

Against this background the material still contains a contradiction. The frequency of ring bivalents is remarkably small as compared with that of multivalents. We must, however, remember that the exchange of partner in itself retards the course of pairing. In a primary multivalent the pairing of each chromosome arm provides an obstacle for the pairing of the neighbouring arm pair. This difficulty is, however, removed if both the arms of one chromosome pair with the same partner chromosome. It is, thus, in agreement with expectation that the unpaired centric region is more often found in multivalents than in bivalents.

Summing up: *The incomplete pairing in the polyploid nuclei, which in general decreases the chiasma frequency and the probability of multivalent formation, increases both of these in the Odonata and in other organisms with chiasma interference across the centromere, in that it interrupts the interference.*

III. GENERAL ASPECTS.

1. THE THEORY OF THE PRIMARY OR OBLIGATORY CHIASMA.

Chiasma interference across the centromere has been concluded to exist in all the cases in which a clear negative correlation prevails in the chiasma formation between the two arms of one chromosome, which should initially apparently have the same chance to form a chiasma. Recently, however, OWEN (1949) has questioned this view and put forward a rival hypothesis to explain the remarkable fact of such a negative correlation. His hypothesis of »a primary or obligatory chiasma» is founded on the assumption (*l. c.*, p. 357) »that the first chiasma to be formed in a chromosome pair, being a necessity for bivalent formation (and therefore an essential prerequisite for subsequent chiasmata), is not on equal footing with chiasmata formed later». On this view the negative correlation in chiasma formation between the two arms can be explained without assuming an interference across the centromere in the cases in which chiasma frequency is very low. OWEN's deduction, which is carried out in purely ma-

thematical terms, is based on an analysis of CALLAN and MONTALENTI's *Culex* and *Theobaldia* cases.

OWEN's hypothesis will not be dealt with in detail in this connection. The attention of a cytologist is attracted by its main idea that the primary and secondary chiasmata are not on equal footing, the former being a prerequisite for the occurrence of the latter. This assumption is, however, justified only in the purely formal sense that the existence of a »2nd» is, of course, always conditional on the existence of some »1st» (!). Cytologically, however, such a relationship cannot be assumed to exist, since no cases are known in which the occurrence of one chiasma could be the cause of the formation of further chiasmata. In the opinion of a cytologist a necessary prerequisite for the formation of a chiasma is furnished exclusively by a sufficiently intimate zygotene pairing at the point concerned. In this sense the formation of all chiasmata must be regarded as being on an equal footing.

This view is corroborated especially by the tetraploid spermatocytes studied in the present paper. The multivalents occurring in them prove that the pairing of each individual arm and the formation of a chiasma in it are independent, autonomous processes. In view of the fact that in a multivalent the two arms of one chromosome form a chiasma with different partners it is, indeed, impossible to assume that the first chiasma in a chromosome is in any sense a prerequisite for the second. Since this situation occurs in regard to multivalents, there is no reason to assume that the same is not true of bivalents. A chiasma can interfere with the formation of another chiasma; it is, however, impossible to think of any other relationship between chiasma formation in the two arms of one chromosome.

Accordingly, OWEN's hypothesis cannot be regarded as having much to recommend itself.

2. CHIASMA INTERFERENCE ACROSS THE CENTROMERE.

Chiasma interference across the centromere has been shown to exist in a number of organisms belonging to non-related groups. This feature is, thus, not restricted to any particular taxonomic unit. In many cases the occurrence of this type of interference is difficult to prove. Only in special cases in which interference is so strong that a chiasma in one arm manifestly prevents the formation of a chiasma in the other can this phenomenon be demonstrated. It is therefore probable that this phenomenon is more common than is supposed at present.

In any case it is clear that the centromere cannot be regarded as an absolute barrier to interference.

On the other hand, we have examples of a chiasma being established in the most proximal regions of both the arms of a chromosome (*e. g.*, in *Allium porrum* and *A. fistulosum*; LEVAN, 1940). In such cases at least, interference does not extend from one arm to another. Now, what is the reason for this divergent behaviour?

In the first place we can assume that in different cases the centromere varies in structure, being in certain organisms transparent to interference, in others again not. It is, however, improbable that differences in centromere structure would occur in closely related organisms such as, for instance, *Culex* and *Theobaldia*, or in *Petunia violacea* and *P. axillaris* (*cf.* above). This idea is further weakened by the fact that in the Odonata the very same centromere would behave differently in the male and in the female. Therefore the conclusion can be drawn that the transparence or non-transparence of the centromere to interference in different species and groups is not determined by any inherent structural differences in it.

As a second possibility we might consider a difference in the division cycle of the centromere. It is not impossible that the reaction of a centromere to interference might depend on whether it is single or double, for instance, in that in the former case it might be non-transparent to interference, in the latter again transparent.

This view cannot be wholly disregarded in the case of the Odonata. The auto-orientation of the centromeres in the first meiotic metaphase supports the idea that the centromeres are perhaps divided even during the diplotene, being thus transparent to the interference at the time when chiasmata are formed. This would, however, hold true only in the male, the bivalents in the female behaving differently. In the oocytes, in which no strong interference across the centromere exists, the centromeres in the bivalents ought to behave as if single in diplotene, whereas at the latest in the metaphase they ought to be double.

In those animals and plants, however, in which the meiotic mitoses take place prereductionally such an interpretation is naturally impossible. Thus, for instance, in *Culex* and other Dipterans the centromeres co-orientate in the first metaphase, which must be regarded as a proof of their still being effectively single at this stage. In consequence, it is naturally impossible that they could act as effectively double bodies as early as diplotene. The inevitable conclusion seems to be that interference is able to penetrate also an undivided centromere.

The practically complete disappearance of chiasma interference between the two arms of a chromosome in a proportion of the multivalents and bivalents in *Ae. s. elisabethae* has been interpreted in the present paper as due to an unpaired region around the centromere of the chromosome, across which interference is not able to proceed. *The same interpretation is in my opinion applicable to the other cases in which the interference does not penetrate the centromere region.* It is well-known that in many organisms pairing is far from complete, even fairly long unpaired regions occurring in the chromosome. If pairing is initiated in both ends of a chromosome, it is obvious that the unpaired region is most probably situated in the middle of the chromosome, in metacentric chromosomes around the centromere. Moreover, it is not impossible that the heterochromatic segments which in many cases lie around the centromere provide an obstacle to intimate pairing, and thus prevent the action of interference across the centromere.

A special merit of the last hypothesis is its great simplicity. On this view the variable behaviour of the centromere in regard to interference becomes a trivial and understandable matter. We are not any longer concerned with the structure of the centromere or its division cycle, but with the variable pairing properties of the chromosomes. This view is corroborated by the fact that chromosome pairing and chiasma formation are processes which often exhibit differences between related species and races, or even between the two sexes of a single species. Differences in these respects occur even between the spermatocytes and oocytes of one hermaphroditic individual, as has been observed by PASTOR and CALLAN (1952) in *Dendrocoelum lacteum*. Thus the variation in chiasma interference may well be correlated with the variable pairing properties of the chromosomes. For the elucidation of this problem, exact and reliable observations concerning the mode of chromosome pairing in different organisms are needed.

The character of chiasma interference is a much discussed cytological problem which ought to be considered in connection with the question of chiasma mechanics. It will not, however, be dealt with in this connection. Instead, a short summary of the facts relating to the mechanism of chiasma interference is given. The following points must be kept in mind when a causal analysis of this mechanism is attempted.

(1) Chiasma interference does not act across unpaired chromosome regions.

(2) The centromere does not in itself provide an obstacle to chiasma interference, since in many cases it has been shown to be transparent to it.

(3) It is most probable that the exchange of partner in multivalent configurations does not necessarily interrupt the interference provided that the successive structural units of the chromosome, say chromomeres, have paired intimately at the very point of exchange.

SUMMARY.

The chromosomes of *Aeschna* species are, like those of other Odonata, metacentric, having arms of equal length. In the spermatocytes at lepto-zygotene bouquets are formed, all the chromosome ends being polarized. In spite of this, a chiasma is practically never formed in more than one arm pair of the bivalent. There, thus, arise only 0,50 chiasmata per chromosome. This almost absolute negative correlation between chiasma formation in the two arms of one chromosome is an indication of an almost absolute chiasma interference across the centromere.

Chiasma formation in a number of tetraploid spermatocytes in *Ae. subarctica elisabethae* has been analysed. In these cells 27 % of the maximum theoretical number of multivalents are realized. Of the bivalents 6,7 % are rings with a chiasma in both arm pairs; the rest are rod bivalents, similar to those found in normal spermatocytes. The chiasma frequency in the tetraploid spermatocytes is relatively higher than in the diploid cells, being 0,63 per chromosome.

This implies a considerably weakened chiasma interference as compared with the diploid cells. In regard to the bivalents the weakening is less, being, however, significant even in them. In the multivalent configurations, again, interference across the centromere has practically wholly broken down, as is seen from the fact that chiasma formation in their four arm pairs is completely at random.

The general theory of multivalent formation in autotetraploids with metacentric chromosomes has been considered in detail. In the light of this discussion the percentage of multivalents in the present case is considerably lower than expectation. The most probable reason for this is the following: In zygotene the expected number of primary multivalents are formed. However, chiasma interference, which acts across the centromere as well as across the point of exchange of partner, allows the formation of chiasmata, as a rule, in only two *inter se*

homologous arm pairs, the two other *inter se* homologous arm pairs remaining without. Consequently, the majority of the primary multivalents fall apart into two bivalents in diplotene. Definite multivalents are obtained only where in a configuration with an exchange of partners, pairing has been hampered and the centric regions of the chromosomes have remained unpaired, which in turn interrupts the interference between the two arms.

It is therefore most probable that the exchange of partner is not in itself the factor interrupting the interference.

In the oocytes the interference across the centromere, if present at all, must be negligible, as practically only ring bivalents are formed.

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CHEMICAL AND BIOLOGICAL EFFECTS OF NEUTRONS AND X-RAYS

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SEVERAL biological effects produced by different kinds of ionizing radiations are the subject of a series of investigations (EHRENBERG *et al.*, 1952, and literature quoted there). For the purpose of drawing conclusions as to the mechanism of action, the effectivities of different radiations have to be compared on the basis of energy absorption in tissue, here called dose. The effectivities can then be related to properties of the radiations.

Of the radiations studied, the fast neutrons present the greatest difficulties as to the dose determination. This is due to the following reasons:

(1) The neutrons dissipate energy in tissue mostly through the projection of light nuclei, chiefly H (about 85 per cent of the absorbed energy is given to recoil protons), C, N, and O, which, when slowing down, ionize the atoms passed. The energy absorption is therefore dependent on the atomic composition of the absorber.

(2) Devices for the production of a neutron beam of sufficiently high intensity give, as a rule, an energetically heterogeneous beam. In our case the neutrons were produced through the reaction Be 9 (d, n) B 10, where the deuterons were accelerated to about 7 MeV in the 80 cm cyclotron of the Nobel Institute for Physics, Stockholm. As the deuterons are slowed down in the 2 mm thick Be target, and as the nuclear energy liberated, *i. e.* given to the neutron, has 5 maxima in the region $-0{,}_{74}$ to $+4{,}_{39}$ MeV (WHITEHEAD and MANDEVILLE, 1950), neutrons of all energies from a low value to about $11{,}_{5}$ MeV are produced. The neutron cross sections, σ, of the atoms being scattered vary in an irregular manner with neutron energy. Some of the atoms (C, N, O) have σ maxima within the actual region of neutron energies (GOLDSMITH *et al.*, 1947; JOHNSSON *et al.*, 1951). As the distribution of neutron energies can only be determined with a fairly large uncertainty, a calculation of the absorbed energy becomes uncertain.

A series of measurements of proton ranges produced in Ilford's nu-

clear research emulsion (type G. 5, thickness 50μ, emulsion placed parallelly to the neutron beam) have given an average proton energy (calculated from the data af LATTES *et al.*, 1947) of 1,57 MeV, corresponding to a neutron energy of 2,48 MeV (*cf.* SIRI, 1949, p. 124). From the composition of the emulsion it can be calculated that about 32 per cent of the recoil nuclei will be C, O, or N. If the 32 per cent shortest tracks are regarded as being produced by these heavier nuclei, the remaining ones will give 2,04 and 3,22 MeV, respectively, for the average proton and neutron energies. A value of 3 MeV for the average neutron energy is used in the following. As to the distribution of proton energies the measurements indicate that most protons are in the region 0,5—2 MeV, but that energies up to about 7 MeV occur.

(3) A recalculation to tissue dose from measurements with a small ionization chamber (ZIMMER, 1938) will be uncertain; and this is also true when the neutron energies are known, owing to incomplete knowledge of the stopping powers of walls and tissue for the recoil nuclei. However, the order of magnitude of the tissue dose can be determined through an application of AEBERSHOLD's (*et al.*, 1942, 1946) quite arbitrarily defined n unit. 1 n is said to equal 2—2,5 rep (roentgen equivalents physical; *cf.* SIRI, 1949, p. 412).

(4) The more correct ways proposed for a dose determination through measurements with an ionization chamber, the walls and gas filling of which have the same atomic composition as the tissue (GRAY, 1944), or where the gas volume can be eliminated through interpolation (FAILLA, 1936; ROSSI and FAILLA, 1950), are liable to great experimental difficulties. Such adhere also to a direct measurement of the dose using GRAY's ν unit.

THE REQUIREMENTS OF A CHEMICAL DOSIMETRY.

The complications summarized, which make measurements of neutron doses uncertain, clearly demonstrate the necessity of a direct determination of neutron doses in some energy unit, *e. g.* rep (1 rep=93 ergs/g of tissue; Nat. Bureau of Standards Handbook 42, 1949) or, which would be equally valuable in radiobiological work, in numbers of ionizations per unity weight of tissue. Preliminarily, the present paper regards the possibilities of using the yields of chemical indicator reactions, occurring in solutions of the same atomic composition as the tissue, as a measure of the dose. Simple oxidations of ferrous iron and of hydroiodic acid have been studied. From a radiobiological point of

view it seems correct to use such effects for a dose determination, as an oxidation of, for example, —SH groups can be demonstrated to occur *in vivo* during irradiation (determined in embryos of germinating barley; unpubl.). — Chemical dosimetries have been applied by several workers (FRICKE and MORSE, 1927; MILLER, 1948, 1950; DAY and STEIN, 1951) and have been shown to give fairly accurate results when all precautions are regarded.

In order that a chemical reaction could be used for the purpose of dose measurements of different types of radiation, the following conditions must be fulfilled:

(1) The reaction yield must be proportional to the energy absorbed per g of solution (or, in any case, to the number of ion pairs produced per unity weight).

(2) The reaction yield must be independent of the nature of the radiation, *i. e.* of its specific ionization (ion pairs produced per μ path) within the region of specific ionizations to be compared. In the case of more densely ionizing radiations as α-rays the reaction yield often decreases on dilution of the solution, due to a recombination of the active radicals formed out of the solvent molecules, before they reach and react with the solute molecules; *cf.* LEA, 1946, p. 57. The specific ionizations of different types of radiation are seen in Table 1.

(3) If the neutron energy absorbed in tissue is to be measured the

TABLE 1. *Ranges and ionization densities of radiations.*

Radiation	Primary ionizing particles	Range[1] in tissue of unit density, μ		Average ion. density of mean energy particles[2], ion pairs/μ
		Maximum value	Effective mean value	
1-11,5 MeV neutrons	protons	700[3]	100	700
	other nuclei	?	?	high
x-rays (165 kV)	electrons	325[4]	5	100
γ-rays (Co 60)	electrons	6000[5]	2000	8
α-rays (Po)	He nuclei	39	39	4500
β-rays (P 32)	electrons	8100[6]	2600	8
β-rays (S 35)	electrons	200[6]	20	51
10 MeV electrons	electrons	about $3 \cdot 10^5$		8
150 MeV protons	protons	about $2 \cdot 10^6$		16

[1] The ranges of the straight paths of electrons are given. — [2] The ionization energies are regarded to be 32,5 eV for all kinds of radiations. — [3] Mean energy protons projected by 11,5 MeV neutrons. — [4] 165 keV photo-electrons. — [5] 1,3 MeV photo-electrons. — [6] The maximum range in water given by SIRI (1949, p. 72).

reaction must be able to occur in a milieu of the same atomic composition as the biological object.

(4) If, on irradiation, the compound A reacts according to: $A \rightarrow B$, it is necessary that A as well as B are sufficiently stable in the solution used.

(5) It must be possible through some simple method to analyse on A or, preferably, B. The reaction yield must be sufficiently large for an accurate analysis at the dose ranges used in biological work.

For a dose determination the reaction mechanism is irrelevant, if there are no reasons to suspect a different mechanism at different types of radiation. Although a very complicated reaction (FRICKE *et al.*; MILLER) the oxidation of ferrous iron has been found to fulfil the requirements (1)—(5) above (*cf.* p. 491).

PROCEDURES. THE COMPOSITION OF TISSUE AND MODEL SOLUTIONS.

As the intensity of the neutrons produced with the device mentioned is not constant, a relative integral dose has been determined for each neutron irradiated specimen in all previous biological experiments (*cf.* GUSTAFSSON and MAC KEY, 1948; MAC KEY, 1951; EHRENBERG *et al.*, 1952). A ring of pure Al was fixed around each test tube containing the seeds, and the amount of Na 24 produced through an (n, α) reaction was determined with the arrangement allowing β—γ coincidence measurements. Na 24 decays, with a half-life of 14,9 hrs, according to the scheme:

$$\text{Na } 24 \rightarrow \text{Mg } 24 + \beta^- + 2\,\gamma.$$

The number of disintegrated Na nuclei per g of Al and per min, recalculated to the mid-point of the irradiation, is used as a relative measure of the neutron dose, called *disintegration*, dis.

The freshly prepared solutions containing Fe^{2+} or some other indicator substance were irradiated simultaneously with x-rays and neutrons and from the reaction yields a relation could be determined, for the solution in question, between the dis unit and the roentgen unit, *i. e.* an absolute measure of the neutron energy absorbed was determined.

The biological investigations have mostly been concentrated on a study of the reactions of germinating or dormant seeds of barley to radiations. As the embryo constitutes the sensitive part of the seeds, the composition of the former is of a greater importance than that of

TABLE 2. *The composition of Bonus barley seeds in per cent of fresh weight. (For C, H, N, the error of analysis is less than 1 per cent, for ash about 5 per cent; the water content is somewhat dependent on the conditions of storaging and pre-soaking.)*

	Dormant Seeds		Germinating Seeds		Water
	Embryos	Total	Embryos	Total	
H	7,40	6,81	9,92	8,26	11,19
C	46,61	41,2	16,90	26,5	
N	4,80	1,50	2,24	0,92	
O	35,25	48,0	68,74	62,8	88,81
Ash	5,94	2,5	2,20	1,55	
H_2O	10	11	66	± 50	100,00

total seeds for an understanding of the reactions producing the radiobiological effects. The composition of dormant as well as germinating seeds (the latter pre-soaked for 24 hrs at 20° C. in 0,1 ml of tap water per seed) are summarized in Table 2. The embryos as well as the total

TABLE 3. *The ash composition of barley seed embryos, in per cent of fresh weight. The value calculated from the data for total seeds of* KOZMINA *and* KRETOVICH *(K. & K.) are compared with data obtained from a direct analysis (E. & N.) of embryos of germinating seeds. These data are also used for embryos of dormant seeds.*

z	Dormant Seeds		Germinating Seeds	
Element	K. & K.	E. & N. (Calculated for 10 % H_2O)	K. & K.	E. & N. (Analysed 71,9 % H_2O)
8 O	2,73		1,20	
11 Na	0,18	0,036	0,07	0,011
12 Mg	0,44	0,37	0,16	0,12
14 Si	0,80	0,67	0,30	0,25
15 P	0,85	0,94	0,32	0,30
16 S	0,07	0,07	0,03	0,03
17 Cl	0,00		0,00	Low
19 K	0,81	1,17	0,30	0,37
20 Ca	0,03	0,05	0,01	0,016
25 Mn		0,0076		0,0021
26 Fe	0,03	0	0,01	0
Others				
Sum	5,94		2,20	

seeds have been analysed. Carbon, hydrogen, nitrogen, and ash were determined according to standard methods, after removal of water at 100° C. Oxygen was taken as difference. For the discussion of the influence of ash constituents on the absorption of radiation energy, the values for total seeds given by KOZMINA and KRETOVICH (1950, p. 141) have been used (Table 3). Lacking closer information as to the composition of the ash at the start of the experiments, we have applied these values also to the composition of the embryo ash. For as the ash is without any noticeable influence on the absorption of neutron energy and only has to be regarded for the determination of the photo-electric, *i. e.* a minor part of the total, absorption of x-rays, we feel entitled to do so.

If the photo-electric absorption of x-rays of all actual wave-lengths is regarded proportional to $Z^{4,1}/A$ (*cf.* LEA, *l. c.*, p. 347), where Z = the atomic number and A = the atomic weight of the absorbing element, an effective atomic number, Z_{eff}, of the ash (exclusive of oxygen) can be computed, if we suppose A = 2 Z. In the calculation the heavier elements headed as »others» are represented by iron. The values of KOZMINA and KRETOVICH give $Z_{eff} = 15{,}8$, *i. e.* the ash elements can with sufficient accuracy be substituted with sulfur. Later an analysis of the principal ash elements in the embryo has been performed. Na, Mg, K, Ca, Mn, and Fe were determined through the courtesy of Prof. H. LUNDEGÅRDH and Dr. I. EKDAHL, by aid of the flame spectro-photometer at the Institute of Physiological Botany, Ultuna, and P and Si were determined spectro-photometrically at 729 mμ as reduced molybdate complexes. P was determined through reduction with metol under conditions free from influence of Si (ALLPORT, 1945, p. 156) and Si through reduction with the same agent in the presence of citric acid (ALLPORT, *l. c.*, p. 156). The data clearly demonstrate a higher relative frequency in the embryo than in the total seeds of heavier elements as P, K, Ca, and Mn, which are known to exert an influence on certain enzymatical processes, whereas the relative concentrations of enzymatically inert elements as Na, Mg, and Si are somewhat lower. The elementary analysis of total seeds performed simultaneously has given data which agree well with those of LUNDEGÅRDH *et al.* (1944), *i. e.* the Na value of KOZMINA and KRETOVICH is found to be somewhat too high, whereas their K value is correspondingly low. The analysis values give a Z_{eff} of 16,4, *i. e.* a somewhat higher value than that calculated on the basis of analysis data for total seeds. Sulfur is still the most correct substitute for the ash elements, and the error introduced will only be perceptible in the case of low voltage x-rays (*cf.* Table 4 and above).

Model solutions can be computed as mixtures of the same number of compounds as the number of elements the concentrations of which are fixed. A fixation of the water concentration will increase the number of compounds with one. The substances have to be stable, also from a radio-chemical point of view: they must not have any preventive effects (as is the case with reducing agents) on the indicator reaction. They must not interfere with the indicator nor with its determination.

Throughout the experiments the iron was given as ferrous ammonium sulfate (Mohr's salt). It was dissolved in 0,1 N H_2SO_4 or in acid model solutions. Different methods were tried for a determination of the small amounts of ferric iron formed during irradiation. The colorimetric determination at 520 mμ with a Beckman spectro-photometer of the thiocyanate complex was found convenient. The titration with permanganate or dichromate of the remaining Fe^{2+} could not be used owing to an interaction of the oxidizing agent with the organic compounds of the model solutions. All substances used in the experiments were analytical reagents. In the case of organic compounds, which were suspected or found to contain interfering impurities, purification and redistillation were performed. Throughout the investigation, the solutions were irradiated in the presence of air of normal pressure.

A mixture containing 0,562 g of water, 0,255 g of glycerol, 0,097 g of acetone, 0,048 g of urea, and 0,036 g of sulfuric acid per g of mixture holds the same atomic composition as embryos of germinating seeds (Table 2). It fulfils all the requirements of a model solution and allows well the determination of Fe^{3+} with KSCN. In earlier experiments (1951) 0,3 ml of 3 N KSCN was added to 3 ml of mixture; in the latter ones, where only extinction coefficients are given (Table 5), 1 ml of KSCN solution to 2 ml of mixture. (The same quantities were used in analyses of Fe^{3+} in 0,1 N H_2SO_4). The density of the mixture is 1,078.

The high carbon and low hydrogen contents of the embryos of dormant seeds bring about certain difficulties for the composition of a model solution. Of course, the problem could be simplified if the nitrogen, which is of minor importance for the absorption of fast neutrons, was excluded. But as there is a certain unknown fraction of rather slow neutrons in the beam, the mixture must have the correct nitrogen concentration. This will render the method useful also for the dosimetry of thermal neutrons, in which case a major fraction of the ionizations are produced by protons, p, formed in the capture reaction

$$N\ 14 + n \rightarrow [N\ 15] \rightarrow C\ 14 + p.$$

A model of embryos of dormant seeds can be composed of water (10 per cent, as in the embryos), pyridine, acetone, acetic acid, and sulfuric acid. In this mixture the ferrous iron is not very stable, but determinations of small amounts of ferric iron can be made within a few hours with thiocyanate, after dilution of the mixture with sulfuric acid and water. Probably the pyridine catalyzes the oxidation of ferrous iron. Far more stable are the oxidation steps of iron in a mixture of 0,118 g of pentamethylenetetrazole (cardiazole, a drug compound with a remarkable solubility in water as well as lipids, in addition to a high degree of stability), 0,100 g of water, 0,103 g of sulfuric acid, 0,281 g of benzyl alcohol, 0,276 g of acetic acid, and 0,128 g of acetone per g of mixture. The density is 1,081. Because of the occurring esterification reaction (formation of benzyl acetate) a mixture in equilibrium, *i. e.* of at least a few hours' age should be used. The determination of Fe^{3+} with thiocyanate is more uncertain, as some reactions obviously start after the addition of this reagent. Before the addition of 1 ml of 3 N KSCN to 1 ml of the mixture, the latter has to be diluted with some lipo-hydrophilic solvent; we used a mixture of 3 ml of 0,1 N sulfuric acid and 2 ml of acetic acid or acetone.

In neutron irradiation, the solutions were kept in thin-walled glass tubes of 6 mm inner diameter. The intensity of the neutron beam will not alter appreciably during the passage through an absorber of this thickness.

X-irradiation occurred of solutions in test tubes of 15 mm inner diameter and a wall thickness of about 1 mm. The radiation energy absorbed in water in such a test tube was determined through a comparison of the reaction yield of Fe^{2+} oxidation (in 0,1 N H_2SO_4) in the tube and in a series of thin-walled, *i. e.* practically wall-less spherical rubber containers (made out of rubber preventives) of different diameters. The roentgen dose rate (air dose) was determined with a 250 r bakelite-walled ionization chamber (Victoreen type, constructed by Dr. MOXNES, Oslo), which was compared for the radiation in question to the standard chamber of the Institute for Radiophysics, Stockholm. The unfiltered radiation from a Coolidge tube run at 165 kV and 6 mA was used throughout the investigation. The correct relation between the reaction yield and the dose (measured in air at the place of the centre of the spheric rubber containers) was obtained through an interpolation of the former to a very small container diameter, at which the intensity of the radiation is not changed during the passage of the solution. The reaction yield in solutions in the test tubes used in

the experimental series was found to be 86 per cent of that obtained in small, wall-less containers. The air dose measured inside a dry test tube is found to be 85 per cent of that obtained at the same place without the glass wall. The dose values (air dose) determined with the chamber inside the tubes can therefore be used, at present with a maximum error of some 3 per cent, due to a variation in the thickness of the glass walls. If a higher accuracy is pursued, this error can easily be eliminated.

The energy absorption in the solutions per r unit air dose is more difficult to evaluate exactly since the spectrum of the unfiltered radiation is unknown. An x-ray tube operated at 165 kV gives a continuous range of wavelengths from 0,075 A (at maximum quantum energy, 165 keV) to about 0,4 A (quantum energy 30 keV). Within this range of wavelengths the photo-electric absorption is dominating at longer wavelengths, especially when heavier atoms are present, the absorption being proportional to $Z^{3,1}$; at shorter wavelengths the greater part of the energy is absorbed by Compton recoil electrons. In the case of monochromatic radiation the energy absorption can easily be computed (*cf.* LEA, *l. c.*). In order to obtain maximum accuracy the relation between the energy absorption and the reaction yield could therefore be calibrated by aid of monochromatic radiation; this has not yet been done.

In order to obtain information about the quality of the radiation a series of air dose measurements were performed with layers of different thickness and of different composition between the x-ray tube and the ionization chamber, and with a great distance between the latter and the tube, *i. e.* giving Compton scattering + Compton absorption + photo-electric absorption. The quality of the radiation was unchanged (observed as a constant absorption coefficient) during the passage through up to 1,5 g/cm^2 of water. The harder elements of the radiation used can therefore be stated to dominate as regards energy absorption in water and the tissues studied. In equally arranged experiments it was demonstrated that the absorption coefficient of the model of embryos of germinating seeds was equal to that of pure water. That of dormant embryos was 1—2 per cent smaller. This is consistent with the absorption coefficients calculated and summarized in Table 4. According to the table, the smaller absorption in the model of dormant embryos indicates a prevalence of the shorter wave-lengths in the actual x-ray spectrum. With a maximum error of a few per cent the energy absorption in water and in the tissues in question for one roentgen unit (air dose) of roentgen radiation can be set 90 ergs/g (*cf.* LEA,

TABLE 4. *Absorption coefficients (μ/ϱ cm g^{-1}) for x-rays, of 3 representative wave-lengths, of materials discussed in the text. Calculated according to* LEA *(l. c.). μ/ϱ of embryos are based on the ash composition data (Z_{eff} = 15,8; vide p. 486) of* KOZMINA *and* KRETOVICH. *As, in reality, Z_{eff} is about 16,4, the absorption coefficients of embryos will be somewhat higher, i. e. the model solutions (Z_{eff} = 16,0) will give slightly low values.*

Absorber	λ = 0,0809 A		λ = 0,1348 A		λ = 0,2022	
	μ/ϱ	H_2O = 100	μ/ϱ	H_2O = 100	μ/ϱ	H_2O = 100
Water	0,02831	100,0	0,02725	100,0	0,03638	100,0
0,1 N H_2SO_4	0,02832	100,0	0,02736	100,4	0,03673	101,0
0,1 N H_2SO_4 + 0,01 N Fe	0,02836	100,2	0,02755	101,1	0,03733	102,6
0,1 N H_2SO_4 + 0,01 N I	0,02912	102,9	0,03082	113,1	0,04771	131,1
Germinating embryos	0,02807	99,2	0,02726	100,0	0,03679	101,1
Model of germ. embryos	0,02807	99,2	0,02729	100,1	0,03690	101,4
The same, without S	0,02790	98,6	0,02641	96,9	0,03409	93,7
Dormant embryos	0,02758	97,4	0,02722	99,9	0,03751	103,1
Model of dormant embryos	0,02760	97,5	0,02732	100,3	0,03782	104,0

l. c., Table 2), *i. e.* 0,97 rep. The uncertainty can be totally eliminated through a comparison of a reaction yield, once for all, with that obtained with monochromatic radiation.

From Table 4 it is further evident that the highest concentration of iron used as indicator, 10 mmoles per 1000 g of mixture, does not affect the energy absorption appreciably. This is also valid for the sulfur of 0,1 N sulfuric acid in water. The importance of the ash content for the total absorption is seen from a comparison of a model of germinating embryos with a mixture containing only H, C, O, and N. The good agreement between tissues and models is also evident from the table.

RESULTS.

The results are summarized in Table 5. In the first instances the amount of Fe^{3+} formed per unit radiation energy and per unit weight of solution was determined through comparison with the colours obtained with standard Fe^{3+} solutions under identical conditions. As a measure of the absolute yield is of no interest for the dose determination, only the extinction coefficients, which are proportional to the

TABLE 5. *Reaction yields and dis/rep relations obtained. The former are given as μmoles per 1000 g of mixture or as extinction coefficients, E.*

Solvent	Reaction yield		No. of dis units corresponding to 1 rep (1 r = 0,97 rep)	
	x-rays	neutrons	Experimental value	Accepted value (*cf.* text)
1951:				
0,1 N H_2SO_4	17,5 ± 2,5 μmol/kr	1,02 ± 0,10 μmol/kdis	18,0	
Model of germin. embryos	25,0 ± 2,5 μmol/kr	1,04 ± 0,04 μmol/kdis	24,8	
1952:				
0,1 N H_2SO_4	0,120 ± 0,020 E/kr	0,0072 ± 0,0005 E/kdis	17,2	18 [1]
Model of germin. embryos	0,090 ± 0,005 E/kr	0,0040 ± 0,0002 E/kdis	23,2	24
Model of dormant embryos	0,0625 E/kr 0,0657 E/kr	0,00138 — 0,00190 E/kdis	35—48	35

[1] Also based on an earlier value of 20,6 and the value of 18 obtained in experiments with iodine.

yield, obtained per unit dose, have been given in the later experiments. The yield per unit dose has been found to vary somewhat from experiment to experiment, due to insufficient uniformity in factors such as the purity of the reagents and the pH, which influence the yield. This difficulty is overcome through a direct comparison of the effects of x-rays and neutrons on the same solutions. This will not be necessary when the methods are settled and the conditions can be sufficiently standardized.

For the germinating embryos a neutron dose of 24 dis is found to correspond to 1 rep, and for water about 18 dis equals 1 rep, where 1 rep is defined as 93 ergs per g. When the experimental errors are considered these values must be regarded as principally correct, for the following reasons:

(1) That the yield of the reaction is independent of the concentration of Fe^{2+} for x-rays as well as for neutrons indicates that the yield is independent of the ionization density (*vide* p. 484). This has been

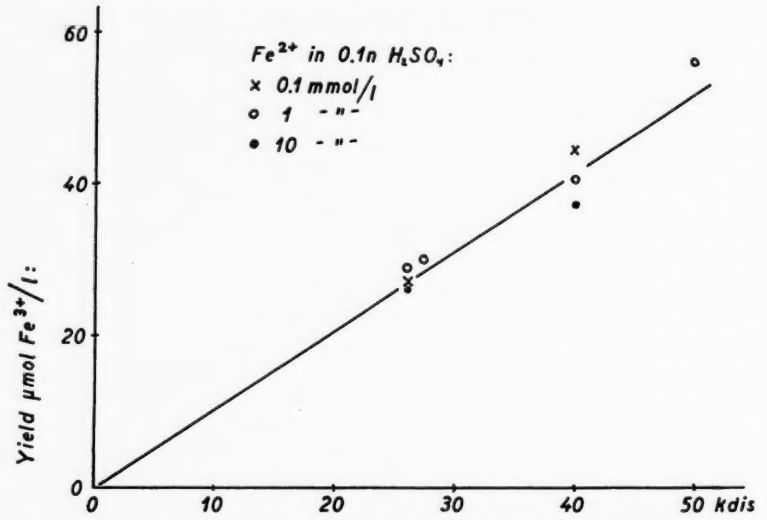

Fig. 1. Yield/dose curve for the oxidation of Fe^{2+} of different concentrations in 0,1 N H_2SO_4 through irradiation with neutrons.

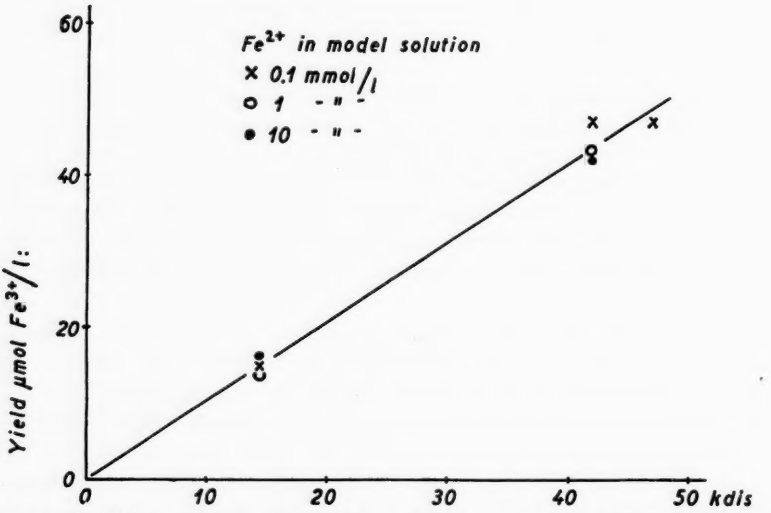

Fig. 2. Yield/dose curve for the oxidation of Fe^{2+} of different concentrations, in model solution of embryos of germinating seeds, through irradiation with neutrons.

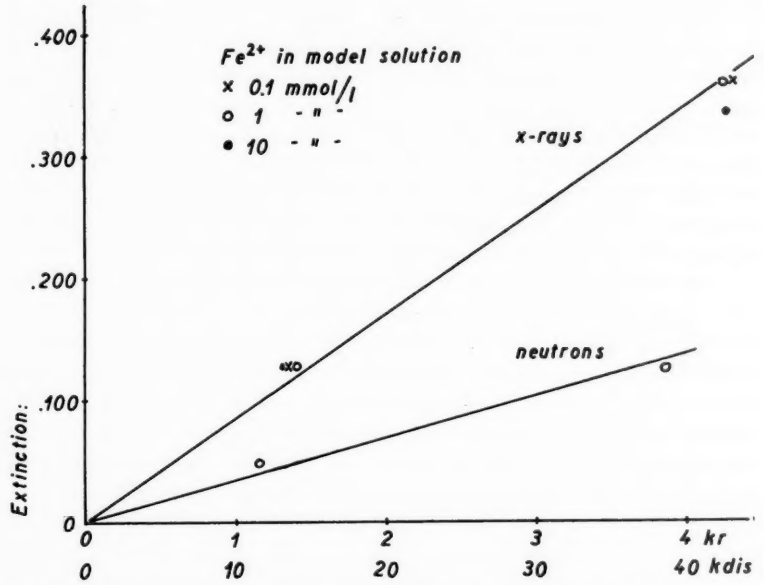

Fig. 3. Yield/dose curves for the action of x-rays and neutrons on the same model solution as in Fig. 2. Extinction values for the ferric thiocyanate complex are given.

demonstrated earlier for the γ — roentgen range of ionization densities (*cf.* Table 1) by MILLER (1950). Nor has any influence been observed of the heavier ionizing nuclei, C, O, and N, the specific ionization of which is certainly much higher than that of protons (*cf.* Figs. 1—3).

(2) The reaction yield of 0,0175 μmol per r and per litre of 0,1 N H_2SO_4 agrees well with the the value 0,016 obtained at pH 1 and in the presence of oxygen by FRICKE and HART (1935). The more complicated reactions found by GRAY and WEIGERT (unpubl.; *cf.* LEA, *l. c.*, p. 46, and MILLER, 1948) in 0,001 N H_2SO_4 are therefore certainly not present. A small dependence of the reaction yield after roentgen but not after neutron irradiation on the concentration of ferrous ammonium sulfate, such that the yield is lower at higher concentrations of the iron salt, has been found in some instances, especially in water but not significantly in the model solutions (Fig. 3). The nature of this dependence is still obscure, but may be related to the reducing power found by FRICKE and MORSE (1927) in freshly prepared solutions. The higher yield in the model of germinating embryos corresponds to a greater acidity.

(3) The magnitude of the values agrees well with a determination of the relation between the dis and n units, performed through an irradiation of a Victoreen thimble chamber surrounded by an aluminium ring. 1 n was found to correspond to about 42 dis (MAC KEY, 1951; EHRENBERG *et al.*, 1952). As 1 n equals about 190 ergs/g of wet tissue (EVANS, 1948, p. 186) about 21 (19—23) dis should correspond to 1 rep. (This measurement is, however, in itself very unreliable. Different condensator chambers with the same wall material but with varying volume give quite different n/dis relations.)

(4) The dis/rep relation for water agrees well with a value obtained with iodine as indicator (*cf.* below).

A few preliminary experiments have been performed with tissue models of the embryos of dormant seeds. Fairly good results have been obtained with x-rays, where large doses are easily produced, but as a suitable method for the analysis of ferric iron is not yet found, only very uncertain values can be given. The low hydrogen content of the solution decreases the effect of neutrons (Table 5) so that only very small amounts of Fe^{3+} are formed in this case. The colorimetric determination with thiocyanate is complicated by the fact that, under the circumstances given, a slow oxidation of the ferrous iron occurs after the addition of KSCN. The uncertainty has also been increased through the presence of a fairly high concentration of ferric iron in the blank, chiefly due to difficulties of obtaining a benzyl alcohol free from oxidizing impurities. The experimental value lies within the limits 35—48 dis per rep. If the value 24 dis per rep be correct for germinating embryos, the lower hydrogen content in dry embryos (Table 2) would give the relation 32 dis/rep, with an uncertainty due to altered concentrations of C, N, and O, *i. e.* in fairly good agreement with the lower limit; the latter will be accepted at present for the calculation of biological effectivities.

One source of error to be mentioned depends on the small size of the embryos, the extension of which is not more than about $1 \times 1 \times 3$ mm. Ionizing particles of fairly long range (*cf.* Table 1) will partly leave the embryo and be substituted by other particles which have been projected in the surrounding tissue (and air), the composition of which is somewhat different (Table 2). For the determination of the x-ray dose the error may be insignificant, since the mean electron energy will be about 15 keV (*cf.* LEA, *l. c.*, p. 12), corresponding to a range of only about 5μ. The protons produced in the neutron irradiation have a mean energy of about 2 MeV (*cf.* p. 482), corresponding to a

range of 75μ (*cf.* LEA, *l. c.*). Owing to the lower hydrogen content of the surrounding tissue, the doses determined will thus be somewhat too high. The error is difficult to evaluate but will probably not exceed a few per cent.

The dis/rep relations accepted at present and given in Table 5 can be regarded as correct within 10 per cent for comparative investigations regarding the relative effectivities of neutrons and x-rays on different subjects. The absolute values of the relation may have a somewhat greater error, due to, among other things, an uncertainty of the r/rep relation. The errors may seem great, but through proper arrangements most of them might, if necessary, be reduced.

In the above calculations the average energy expanded in the production of one ionization has been regarded the same for different types of ionizing particles. In air the ionization energy is 32,5 eV for electrons, whereas it is somewhat higher (35 eV) for protons and α-particles (*cf.* LEA, *l. c.*, p. 22; GRAY, *l. c.*). If there is a similar difference in the ionization of tissue (which is not known), the present dosimetry will measure the number of ionizations instead of the energy absorption. From a radio-biological point of view a knowledge of the former will be the more valuable. Further, the difference between the two magnitudes will not be great, as the electron, as a rule, is the chief ionizing agent: in the case of 2 MeV protons or Po α rays about two thirds of the ionizations are due to secondary electrons (δ rays).

EXPERIMENTS WITH OTHER RADIATIONS.

The β-radiating isotope, S 35, was used in one series of experiments. The isotope material was given as sulfuric acid to a solution of 1 mmol of Fe^{2+} per litre of 0,1 N H_2SO_4. Irradiation times were extended over 8 days. The yield was found to be 0,0163 μ mol Fe^{3+} per 1000 g of solution and per rep. This value is in good agreement with that obtained with x-rays (Table 5) regarding the fact that the activity of the S 35 sample was known with an accuracy of 10 per cent. In the dose calculation the mean energy of the β particles was regarded to be 0,033 MeV (HENRIQUES *et al.*, 1946). In the case of more far-reaching rays, the energy outside the object has to be taken into consideration. If in the case of S 35 the yield is somewhat low it may depend on adsorption to the walls of the tube containing the solution.

Integral tissue doses within the range 2 000—20 000 rep in irradiation of seeds with 10 MeV electrons produced with the synchrotron

of the Department of Electronics (R. Institute of Technology, Stockholm, Head: Prof. H. ALFVÉN) have been determined through the oxidation of ferrous iron. The values obtained agree within 15 per cent with those estimated from a dose rate determination with a 250 r ionization chamber. Since the electron beam produced with the device is variable with time, the determination of an integral dose is necessary. In our experiments small ampoules containing 0,5 ml of test solution (+ 0,2 ml of air) were placed among the seeds of the sample to be irradiated. A similar set of irradiations with x-rays were performed simultaneously.

Doses of 150 MeV protons, produced with the synchro-cyclotron at the Institute of Physical Chemistry, University of Uppsala, have also been determined with the iron reaction.

In irradiations with fast neutrons or protons the irradiated seeds have been subjected to the influence of the magnetic field of a cyclotron. The paramagnetism of the ferrous iron could possibly influence the reaction yield. This influence is, however, negligible, as in the experiments with fast neutrons the diamagnetic indicator, HI, gives about the same dis/rep relation, and in the experiments with fast protons, the dose determined with the iron indicator equals that determined through the degree of oxidation of thiourea which is diamagnetic. In the case of protons a dose determination by aid of the reduction of ceric cerium (*cf.* CLARK and COE, 1937), or the oxidation of hydrogen sulfide have also given congruent results; however, the analysis methods for the latter two compounds have allowed a less degree of accuracy. Thiourea was determined spectrometrically at 235 mμ, Ce^{4+} through titration with Fe^{2+} with iron-orthophenantroline and H_2S through the addition of excess iodine which was determined colorimetrically with starch at 580 mμ.

The oxidation of ferrous iron has been used previously for the determination of doses of γ radiation (*cf.* MILLER, 1951). Owing to the long range of secondary electrons geometrical factors have to be regarded. The dose determined through the oxidation of iron in a thin-walled test tube of 8 mm diameter is about 20 per cent higher than that determined with an ionization chamber, the wall diameter of which is about 5 mm.

In irradiation with α rays there are indications of a dependence of the reaction on the concentration of the indicator iron (NURNBERGER, 1934; *cf.* LEA, *l. c.*, p. 57), *i. e.* there may be a certain recombination of radicals instead of their reaction with Fe^{2+}. In such instances a

chemical dosimetry must include a correction for this effect, *i. e.* the specific ionization of the radiation in question must be known as well as the dependence of the reaction yield on the specific ionization.

EXPERIMENTS WITH OTHER INDICATORS.

In a series of experiments ascorbic acid, determined through titration with dichlorophenolindophenol, has been tried as indicator for dose measurements. It is easily destroyed, or oxidized, through indirect radiation effects. The yield is, however, influenced by unknown factors (impurities?); it is especially low in the tissue models used, and its application for a neutron dosimetry under the present conditions is therefore less convenient.

The neutron dose in water (*i. e.* 0,1 N H_2SO_4) was determined through the measurement of the oxidation of iodide (10 mmoles of KI/l) to iodine. The iodine formed was determined colorimetrically at 580 mμ with maize starch glycolate (0,05 per cent in the total volume). The obtained value of 18 dis per rep is in good agremeent with those obtained with the iron reaction. The dependence of the reaction yield on the iodide concentration and the pH indicate that HI is the compound oxidized (*cf.* LANNING and LIND, 1938). Owing to the high atomic weight of iodine there may be some difficulties in evaluating the dose of polychromatic roentgen radiation (*cf.* p. 490 and Table 4). Since free iodine reacts with acetone, which is a component of the tissue models tried, iodide cannot be used as indicator substance in connection with these. The determination of iodoacetone formed has not yet been tried.

As regards experiments with thiourea, hydrogen sulfide and cerium, see the previous section.

BIOLOGICAL CONSEQUENCES.

The possibility of a determination of the neutron doses in germinating and dormant seeds of barley makes a discussion on an exact basis possible of the enormous differences observed earlier (GUSTAFSSON and MAC KEY, 1948; MAC KEY, 1951; EHRENBERG *et al.*, 1952) between the biological action of neutrons and that of other types of radiation. Table 6 comprises a comparison between the biological activities of x-rays and neutrons, regarding their effects in several respects on barley. Most data are taken from EHRENBERG *et al.* (1952, and

TABLE 6. *Biological effects of x-rays and neutrons.*

Biological reaction produced by the radiation	Dose necessary to produce the reaction, rep			
	X-rays		Fast neutrons	
	Dormant seeds	Germinating seeds	Dormant seeds	Germinating seeds
50 per cent chromosome disturbances in the first mitotic divisions	12500	2500	600	150
50 per cent lethality in the field	15000	2000	350—500	100—200
50 per cent decrease of plant height (see text)	12500	2000	350	150
50 per cent plant sterility	The plants do not survive		700	200
20 » » » »	9500	7000	70	50
2,5 per cent chlorophyll mutations per spike progeny	~ 4000	~ 4000	25—75	18—40

sources given there), but the neutron doses are recalculated in accordance with the values obtained with the iron dosimetry of the present investigation. The table clearly visualizes the greater biological effectiveness of neutrons compared with x-rays in this material. Regarding physiological reactions as lethality and inhibition of growth the difference between the two radiations may be expressed by a factor of about 20, whereas in reactions including chromosomal rearrangements (chromosome fragments and bridges, sterility) and mutations the difference is still greater. Certainly, part of the difference in the latter respect is due to an elimination of chromosomally injured cells during the growth of the plants, which occurs more frequently after roentgen irradiation (especially of germinating seeds) than after neutron irradiation (*cf.* MAC KEY, 1951; EHRENBERG *et al.*, 1952). In the table this elimination process is strongly visualized by the increase of the difference between the two kinds of radiation from about 20 times at the first mitotic divisions in the root to more than 100 times at the time of harvest. The elimination is demonstrated in the diagrams of EHRENBERG *et al.* (*l. c.*) and is also indicated by the mutation experiments of 1952, not included in Table 6. In this year the effects of low doses of x-rays were investigated in order to avoid the elimination effect. In dormant seeds the old values were principally repeated, but in ger-

minating seeds no less than 6,31 per cent of chlorophyll mutations were obtained at 625 r; at a dose of 1250 r only 1,59 per cent of mutations were found. Although unusual conditions in the field make these values less appropriate for comparisons, it is clearly indicated that when the elimination is regarded, the real difference in effectiveness between x-rays and neutrons as to the production of mutations and sterility is smaller than expressed in Table 6, probably the same as for the production of chromosomal rearrangements in the first mitotic divisions.

In this comparison of the biological effectivities the doses are determined on the basis of a simple chemical reaction, demonstrated to be independent of the specific ionizations of the radiations. It is therefore logical to conclude (and this has often been done; *cf.* LEA, *l. c.*) that the yields of the more complex chemical reactions behind the biological effects observed are functions of the specific ionization, the more densely ionizing radiations being the more effective. The conclusion is strengthened by the fact that P 32 β rays, which give the minimum ion density of about 8 ion pairs/μ of tissue, are less effective than roentgen rays in producing most of the effects mentioned (EHRENBERG *et al.*, 1952: Tables 2—4), especially when the absence of elimination of injured cells in the case of P 32 is regarded. As to the growth inhibition, it is more difficult to determine the dose of P 32 radiation, but 10 MeV synchrotron electrons as well as Co 60 γ rays, which give the same ion density in tissue as P 32, have turned out to be about half as active as x-rays in this respect (experiments still going on).

In connection with the different biological effectivities of x-rays and neutrons, a preliminary set of experiments with proteins may be mentioned shortly. Solutions of the enzyme, urease, were irradiated with the two types of radiation and analysed on enzymatic activity. When the dose determination was based on the iron reaction, the effectivity of neutrons was found to be about 5 times that of x-rays. The destruction of the enzyme activity depends on an oxidation of —SH groups (BARRON, 1948), and the enzyme is very sensitive to ionizing radiations. Since most biological effects are due to alterations in proteins, further research on solutions of such will explain why neutrons have a greater effect than less densely ionizing radiations.

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SUMMARY.

(1) Tissue doses of ionizing radiations in embryos of barley seeds have been determined with a chemical dosimetry, involving an indicator reaction occurring in a solution of the same atomic composition as the tissue.

(2) With this arrangement the roentgen equivalents of doses of fast neutrons have been determined. It is proved that for the radiations in question the yield of the indicator reaction (chiefly $Fe^{2+} \rightarrow Fe^{3+}$) is independent of the specific ionization. Doses of β and γ radiation and of fast protons have been determined, too.

(3) The radiation induced oxidation of HI, H_2S, thiourea, and ascorbic acid, and the reduction of Ce^{4+} have also been investigated.

(4) On the basis of the chemical dosimetry developed it can be stated that fast neutrons are at least 20 times more effective than x-rays as regards the production of lethality, growth retardation, chromosomal rearrangements, sterility, and gene mutation, consequent upon the irradiation of dormant or germinating seeds of barley.

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ABSTRACTS

K. G. LÜNING: The connection between chromosome breaks and apparent gene mutations in *Drosophila melanogaster*.

It was demonstrated (LÜNING, 1952 a) that when irradiating males of *Drosophila melanogaster* more breaks were induced in spermatozoa inseminated 7—10 days after treatment than in those inseminated the first 6 days. The rates of apparent gene mutations (*yellow, white* and *singed*) did not, however, vary as the rates of breaks, if there were any variations at all. In order to establish a possible minor variation in the rates of apparent gene mutations (visibles and recessive lethals) induced in various stages of spermiogenesis, further experiments were carried out. For the details, see LÜNING, 1952 b.

In experiments where visible mutations (*yellow* and *white*) were looked for, 0—1 day old males of a wild type stock (Oregon) were irradiated with 960 r and immediately mated to *y w sn* females (in the first part of the experiment) or to *y* sc^{S1} *InS* w^a sc^8, *M5ry* females (in the latter part of the experiment). By the 7th day the males were transferred to new virgin females with which they were left until the 11th day when they were again transferred to new virgin females. They were discarded at the 18th day after treatment. The female offspring from these matings were examined and counted and all *yellow* and *white* mutants were listed. In Table 1 are presented the combined results in the three mating periods.

Comparing the rates of *yellow* mutations in the two periods 1—6 *versus* 7—10 days after treatment, one observes that no difference is established. The rate of *white* mutations is, however, significantly higher in the period 7—10 days than in 1—6 days after treatment; $P < 0{,}001$.

TABLE 1.

Period of insemination in days after treatment	Total female offspring	yellow		white	
		Abs. nr	per 10^4	Abs. nr	per 10^4
1—6	293221	18	0,61	21	0,71
7—10	234276	15	0,64	42	1,79
11—18	102444	3	0,29	1	0,10

The rates of recessive lethals induced in the *y w sn* chromosomes in various stages of spermiogenesis were studied by the Muller-5 technique. In Table 2 are presented the results from three series given 1440 r or 4320 r. A column is included giving the corresponding rates of dominant lethals as an indicator of the rates of the chromosomes breaks.

Comparing the rates of recessive lethals in Ser. 148 *versus* 149, one finds that the latter is significantly greater than the former; $P < 0{,}001$. In Ser. 149 *versus* 159 the rate of recessive lethals in the former is significantly lower than in the latter, $0{,}01 > P > 0{,}001$, in spite of a higher rate of breaks as is indicated by dominant lethals.

TABLE 2.

Series	Dose	Period of insemination in days after treatment	Total sperms tested	Recessive lethals		Dominant lethals in %
				Abs. nr	%	
148	1440	1—6	2967	88	2,97	28,0
149	1440	7—10	2820	145	5,14	74,4
159	4320	1—6	762	64	8,40	65,4

BELGOVSKY (1938) demonstrated that the high rate of *yellow* (and *achaete*) mutations induced in sc^8 and sc^{S1} chromosomes was due to minute re-arrangements. This is an excellent means of studying whether the rates of apparent gene mutations, due to intergenic changes, vary as the rates of breaks in various stages of spermiogenesis. In order to examine if this is the case sc^{S1} *B InS* w^a sc^8 (Muller-5) males were irradiated with 960 r and 3840 r and subsequently mated to *y w sn* females. One control series was also made. The results are presented in Table 3.

TABLE 3.

Series	Dose	Period of insemination in days after treatment	Total female offspring	yellow		hyperploid males	
				Abs. nr	per 10^4	Abs. nr	per 10^4
152	Control		39284	1		1	
137	960	1—6	21647	8	3,7	4	1,8
138	960	7—10	15911	31	19,5	11	6,9
155	3840	1—6	4330	12	27,7	4	9,2

Comparing Ser. 137 *versus* 138, one finds that they are significantly different; $P < 0{,}001$. The ratio between the two rates of yellow mutations is about 5 (the corresponding ratio of the rates of breaks is about 4; LÜNING, 1952 a). On the limited material it was not possible to establish any significant difference between Ser. 138 and 155. One may consequently conclude that the rates of intergenic changes vary as the rates of breaks. As neither *yellow* and *white* mutations in the wild type chromosomes nor recessive lethals vary in this manner, one may conclude that they are not only due to intergenic changes, but also to intragenic changes, and that the latter are not — or only slightly — subject to variation with the stages treated. Thus, one is forced to assume the existence of two types of elements in the chromosomes, *viz.* invariable (or possibly slightly variable) and variable elements. Hits in the invariable elements may produce breaks or other changes; in any case they seem in themselves to induce apparent gene mutations — intragenic. Hits in the variable elements produce breaks which seem in themselves not to induce apparent gene mutations but may do so as a result of a re-arrangement — intergenic.

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BERTIL RASMUSON: Evidence of an autonomous cytoplasmic system in *Drosophila melanogaster*, influencing the resistance to ether.

One selection line of certain bristle characters of *Drosophila melanogaster* proved much more sensitive to ether narcosis than the other lines. An almost homozygous wild-type strain (Florida) was used for genetical comparisons. (RASMUSON, 1951.)

Several lines of backcrossing have been made from the F_1 generation after reciprocal crosses between these two strains. Only two are to be mentioned in this connection. In all experiments 20 females — 24 ± 3 hours old — have been anaesthetized together with 20 females of the same age from the pure sensitive strain, and the differences between the two lethal times are collected in column 3 in the table (Table 1). The ether dose has constantly been about 30 %.

TABLE 1.

Types	Number of exp.	Excess of lethal times over sensitives in sec.	s^2 between exp.	P for differences from sensitives
pure line				
+	11	+ 68,549	159,103	< 0,001
F_1				
+ × S	10	+ 119,571	457,136	< 0,001
S × +	11	+ 94,015	331,917	< 0,001
backcrosses				
(+ × S) × S				
gen. 1—10	22	+ 32,619	321,436	< 0,001
(S × +) × S				
gen. 1	6	+ 46,468	83,218	< 0,001
» 2	6	+ 19,943	215,506	0,05 — 0,02
» 3	6	+ 6,685	94,345	0,2 — 0,1
» 4	6	+ 3,083	19,999	0,2 — 0,1

The cross sensitive mother to normal father (S × +) gives daughters significantly more sensitive than the reciprocal cross (+ × S). As the daughters after these reciprocal crosses have identical genotypes but different cytoplasms, the difference must depend on a maternal effect. Besides this fact the F_1 daughters show a heterosis effect which considerably increases the resistance of both crosses.

In the series of backcrossing (S × +) × S the sensitive genome is reintroduced into the sensitive cytoplasm. After 3—4 generations this series has reached about the same sensitivity as has the pure sensitive strain.

In the backcrosses (+ × S) × S one should also expect the curve to decline to zero after some generations, if the differences in sensitivity only depended upon the genome. However, this series of backcrossing soon sta-

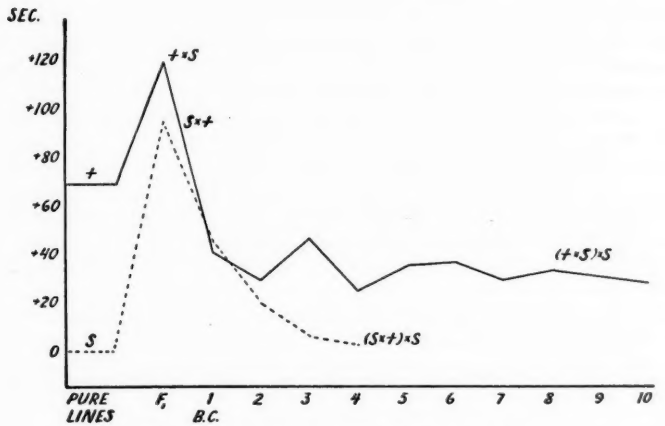

Fig. 1. For explanation, see text and table.

bilizes itself at a lethal time about 33 sec. longer than the pure sensitive strain (Fig. 1). After 20 generations of backcrossing this difference has not disappeared, suggesting that the normal cytoplasm has not been transformed to a sensitive one by the sensitive genome. The differences of resistance to ether — and some other tested narcotics — between these two strains thus seem to depend partly on an autonomous cytoplasmic system and partly on a genome system.

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ARNE HAGBERG: Heterosis in some crosses between populations of rye and red clover.

In a study of the occurrence of heterosis, crosses have been analysed, on the one hand between pure lines of self-fertilizing species as *Galeopsis* (HAGBERG, 1952 a and b) and barley (HAGBERG, 1952 c), and, on the other, between populations of two cross fertilizers, rye and red clover. In the breeding of cross fertilizers the heterosis effect is often utilized by crossing lines, strains or even populations which have been more or less inbred or at least differentiated, and by distributing F_1 seed to the farmers. The stricter the inbreeding the more this method approaches the crossing of pure lines of self fertilizers; such is the case, *e. g.*, in the production of »hybrid corn» in maize breeding. In many plant species, such as sugar beet (ASHTON, 1946, and others) and rye, strict inbreeding does not appear to be applicable in practical breeding work; other species, as red clover, have a system of self sterility alleles (WEXELSEN, 1945) which make it impossible to use strict inbreeding. In some of these species, however, the crossing of differentiated and more or less inbred populations has been used with good results.

It seems to be of interest, therefore, to study more closely the effects of crosses of this type. MÜNTZING (1943) has shown that double crosses between inbred strains of rye are superior in yield to the population from which the strains were derived, but it is difficult to find a method for utilizing this fact in the breeding work. PETERSEN (1934) is of the opinion that it is possible to produce F_1 seed of rye by mixing a number of inbred strains, while HEUSER (1935) considers it impracticable to utilize heterosis in practical rye breeding.

Several authors (CRÉPIN, 1928; BREDEMANN and HEUSER, 1931; SCHRIBAUX, 1931) found an increase in yield when the seed from a mixture of two varieties was used.

The experiments with rye. — Crosses have been made between different populations of spring rye. The spring form was used in order to avoid the variations caused by differences in winter hardiness. The varieties used have been: (1) Petkus, a high-bred German variety, most commonly in use in Sweden; (2) a Swedish land-variety from Od in Västergötland, obviously different from Petkus; (3) a variety obtained under the name of »Giant spring rye» from Sjöbo in Skåne (Scania), which variety is very similar to Petkus. The first two varieties were obtained from the Rye Department of the Swedish Seed Association at Svalöf, the third one from the Weibullsholm Plant Breeding Station, Landskrona. The parent populations were sown in 1945 and 1946, and crosses made in both years.

Two rows with spaced plants were sown of each variety to be used in a cross:

Row A ——————————
variety P_1
B ——————————
C ——————————
variety P_2
D ——————————

One ear from row B was isolated in a pergamin bag together with an ear in the same stage of development from row C of the other variety. Another ear from the same plant in row B was isolated together with an ear from row A, and one ear from the plant in row C with an ear from row D. These crosses correspond to the »pair crossings» of HERIBERT NILSSON (1937). In this way about 100 crosses were made between varieties and a corresponding number between plants within the same variety. For the comparisons between the different combinations the seeds harvested from the plants in rows B and C have been used.

The seeds were sown by marking board in rows 15 cm apart, 15 plants to the row and 10 cm between the plants in the row. All plants in a row belonged to the same combination. For each of the three possible crosses there are two intra-parental combinations and two reciprocal inter-parental ones. In the cross Od × Giant the combination Giant × Od gave too few seeds in 1945 to allow for an adequate comparison, and this combination is excluded from the material. The rows, each with one combination, are replicated, and the replicates distributed in a repeated 4 × 4 Latin square as described for the *Galeopsis* material (HAGBERG, 1952 a).

The material was closely followed during summer, but there were no apparent differences between the P and F_1 populations in regard to earliness, determined as the day of heading, or in the general rhythm of development. After the harvest each plant has been weighed and also the plant yield of grain. Further, the length of the tallest straw has been determined and the length of the ear on this straw. The tillering has been measured by the number of ears per plant and the seed setting by the number of seeds in per cent of the number of flowers. Finally, the pollen fertility has been estimated by the percentage of apparently good pollen in Belling-glycerine preparations.

In 1946 the material was very well developed and fairly reliable results were obtained. In 1947, spring and early summer were extremely dry. The germination was uneven and also the further development, and the material of this year was not very suitable for investigation of this kind.

Table 1 gives the data from the tests of 1946. The analysis of variance in Table 2 reveals a significant difference between populations in yield of grains and also shows that the difference between F_1 and the highest yielding parent line was highly significant. A further analysis of the data in Table 1 shows that in the combination Petkus × Od the yield of F_1 is about 12 % superior to that of Petkus. In the combination Giant × Od F_1 yields 17 % more than Giant, while in Giant × Petkus F_1 is only 2 % superior to the best of the parents. As already mentioned, Giant and Petkus are rather similar, and it seems probable that they are more or less closely related. (Giant may simply be a Petkus grown at Sjöbo for some years, the original name having been forgotten and a new one given, a phenomenon far from unknown.) The land variety from Od is distinctly different from both the others. The obvious assumption is that a close relationship between Giant and Petkus is the cause of the low degree of heterosis in the cross between them, and the data indicate an increase in inter-population heterosis with an increase in the genetical difference between the populations.

TABLE 1. *Results from measurements on plants of F1's and parent populations of spring rye. 1946.*

Population	Number of plants	Grain weight g/pl.	Plant weight g/pl.	Number of ears/pl.	Length of straw cm	Length of ear cm	Seed setting %
Petkus spring rye	266	5,64	13,79	4,88	126,4	8,00	81,7
Petkus × Od	276	6,05	14,00	5,03	127,7	8,70	80,0
Od × Petkus	265	6,58	15,36	5,48	128,3	8,88	81,0
Rye from Od	256	5,07	12,21	4,83	127,0	9,14	79,1
Petkus	203	5,14	14,22	4,45	127,7	7,61	81,1
Petkus × Giant	225	5,39	14,60	4,79	128,8	7,97	81,1
Giant × Petkus	215	5,11	14,30	4,46	130,5	8,14	81,0
Giant	229	4,81	13,84	4,82	130,2	7,98	77,7
Od	225	5,28	12,45	5,15	125,7	9,59	76,4
Od × Giant	228	6,27	14,70	5,37	127,7	9,02	78,4
Giant	242	5,36	13,86	4,91	129,9	7,93	78,2

TABLE 2. *Analysis of variance of data concerning grain yield per plant.*

Cause of var.	DF.	Sum of squares	Variance	Quotients
Total	2631	19946,85	—	—
Between populations	10	733,63	73,36	$10,91^{***}$
Between F_1 and Pmax	1	110,69	110,69	$15,10^{***}$
Within populations	2621	19213,22	7,33	—

TABLE 3. *Percentage of good pollen in the cross Od × Giant.*

Population	1946	1947
Spring rye from Od, Västergötland	$89,3 \pm 1,2$	$90,0 \pm 1,1$
Giant rye from Sjöbo, Skåne	$90,9 \pm 1,0$	$91,5 \pm 1,2$
F_1	$89,9 \pm 1,0$	$89,2 \pm 1,4$

It is remarkable that there is no indication of a heterosis in the length of the straw but only in tillering and, consequently, in weight of plant and yield of grain. The fertility is good in F_1, as a rule as high as in the most fertile of the parents. This is true of the seed setting (Table 1) as well as of the pollen fertility. Table 3 gives the data from one cross, representative for the whole material.

TABLE 4. *Results from measurements on plants of F1 and parent populations of spring rye. 1947.*

Population	Grain yield g/pl.	Plant weight g/pl.	Number of ears/pl.	Length of straw cm	Length of ear cm
Petkus	4,1	9,7	3,3	106,7	7,1
Od	4,5	10,4	3,9	112,9	9,3
Petkus × Od + rec.	4,9	11,2	3,8	112,0	8,7
Petkus	4,1	9,7	3,3	106,7	7,1
Giant	4,8	11,7	3,7	115,2	7,8
Petkus × Giant + rec.	4,9	11,7	3,6	113,8	7,8
Od	5,0	11,5	4,3	108,9	9,4
Giant	4,7	10,9	3,8	116,1	7,6
Od × Giant + rec.	5,3	11,7	4,3	111,3	8,7

Table 4 presents the results of the 1947 tests. The material was poorly developed in this year, but the data are, nevertheless, presented, since on the whole they support those of 1946.

Some experiments were carried out with winter rye in 1946—48. In 1946 the Svalöf variety Stål was crossed with the German Ringroggen; in 1947 the two Svalöf varieties Kungs II and 0801b were crossed. The material of the latter cross was accidentally destroyed in 1948, that from the former combination grew through the very severe winter of 1946—47 and the following dry spring of 1947, and was severely damaged. The yield of grains per plant was higher in F_1 than in parent populations, 6,27 against 5,93 grams, but the P-value of the difference is 0,05.

The experiments with red clover. — Eight local strains of red clover, all obtained from the Herbage Department of the Swedish Seed Association, were used, *viz.*: Karaby and Harrie from the province of Skåne (South Sweden); Hassle-Säby from Västergötland, Sörby-Okna from Östergötland, Ultuna from Uppland, and N. Edsberg from Värmland (all Central Sweden); Offer from Ångermanland and Kusträsk from Norrbotten (Northern Sweden). The clover material was sown and planted in the field in 1945, and in 1946 crosses were made, using 10 plants of each strain, selected at random. The Harrie strain was used as mother in crosses with the other seven strains. Within each strain one of the plants used in the inter-strain crosses was used as father plant for pollinations of the other nine ones. The seed was sown and planted in the field in 1947. Careful observations were made on general vitality and other characters; and in 1948 the plants were individually harvested, the weight of green matter being determined and the regrowth studied.

No significant differences between the inter-strain and intra-strain crosses have been found in a thorough statistical analysis of the data and I refrain from presenting these in detail. Even negative results are often of considerable

value. It is known that the local strains are rather adaptable, if grown under conditions other than those prevailing in the district where they have originated through natural selection. And it is reasonable to assume that the genetical variation within the strains is still very extensive, even in comparison with the differentiation between strains.

Summary. — On crossing differentiated populations of rye, very slightly inbred and with a comparatively restricted number of alleles, a heterosis effect has been found in yield of grains, in comparison with crosses between plants within the populations. There is an indication that the degree of heterosis is parallel to the degree of genetical differentiation between the populations. Crosses between different populations of red clover have not shown a similar heterosis effect, which is hypothetically explained by the fact that the genetical variation within those populations is probably greater, in comparison with the differentiation between populations, than is the case in the rye populations studied.

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A. HAGBERG, N. NYBOM and Å. GUSTAFSSON: Allelism of erectoides mutations in barley.

Since the work with induced mutations began in Sweden more than two decades ago, a great number of mutants have been isolated in various crop plants (*cf.* GUSTAFSSON, 1947; GUSTAFSSON and MAC KEY, 1948). Among the barley mutants, fully viable and promising from a practical point of view,

the so-called erectoids represent the most frequent mutation type. All of them are characterized by compact, dense ears, implying that the ear internodes are shorter than in the mother strain. In addition, they generally possess a very stiff and often also short straw. In the course of years about sixty such mutations have been detected, 47 of which are now registered and the subject of continuous research. Ten are still in the second or third mutant generation.

The practical evaluation of these mutations would gain much in interest, if their genetical and cytological nature could also be clarified. A few data on allelism and linkage have been published previously (NILSSON-EHLE, 1939; GUSTAFSSON, 1947, and NYBOM, 1950). Recent studies have revealed interesting cytological properties of some erectoides mutants (HAGBERG and TJIO, 1950; TJIO and HAGBERG, 1951). A general survey is however missing. This brief article is planned to fill some gaps in our knowledge of their behaviour.

Diallelic crossings have been carried out between a great many erectoid mutants, divided into separate groups after their strain of origin. Up to now at least 14 different loci have been found, which after mutation exert a conspicuous influence on ear density. One locus is more mutable than the others. No less than eight out of 28 tested mutations are situated at this very locus. The eight mutations are *ert* 6, 11, 13, 19, 21, 23, 28, and 29 (as to the marking *ert, cf.* HAGBERG and TJIO, 1952). In another locus four cases of mutation have been obtained. They are *ert* 7, 14, 15, and 33. By cytological means this locus has been fixed as to its situation, for *ert* 7 lies close to or at the very point of a translocation (HAGBERG and TJIO, 1950). In addition, we can state that *ert* 14 is similarly connected with a translocation, but *ert* 15 and 33 are not.

A third locus mutated at least three times, viz. *ert* 2, 4 and 5. Possibly *ert* 9 belongs here, too. The mutant strain, however, registered under this name, is heterogeneous and as yet not definitely analysed. In a fourth locus *ert* 1 and 39 are situated, the former one being closely linked to a translocation point (TJIO and HAGBERG, 1951). The remaining ten mutants represent different loci.

Eight erectoides mutants out of Golden barley (Gullkorn) were the subject of the cross analysis first undertaken. The F_2's of the diallelic crossings were bred in 1951. The mode of segregation indicated that the locus of *ert* 2, 4 and 5 is linked to that of *ert* 7. The other crossings showed free recombination of the erectoides factors. Linkage studies of the remaining twenty and more mutations are on the way.

The following problem appears to us to be specially important: Are the mutations of one and the same locus identical or do they produce differences detectable by morphological or statistical analysis? In other words: Do identical changes always take place within one and the same locus, or, on the contrary, do all mutations of a locus represent different alleles? Table 1 contributes to an analysis of the problem. The ear density, defined as the length of ten internodes from the base of the fifth kernel upwards, has been measured for a series of F_1's between mutations of a single locus, the most mutable one. One of the mother strains, Bonus barley, a common commercial nutans barley, has a value of the ear density amounting to $32{,}1 \pm 0{,}16$. The standard errors of the values of the table range between $0{,}08$ and $0{,}20$.

TABLE 1. *The values of ear density in an allele series of erectoides mutants.*

Ert	6	11	19	21	23	28	29
29	—	20,1	21,5	20,9	20,0	23,3	*19,8*
28	—	22,6	24,4	24,9	—	*25,6*	
23	—	20,1	21,0	21,0	*21,3*		
21	—	20,9	21,6	*23,1*			
19	—	20,1	*20,8*				
11	19,4	*19,3*					
6	*19,8*						

Consequently the individual mutations show pronounced differences as to their phenotypical expression. For instance, the ears of *ert* 6, 11 and 29 are extremely dense. On the other hand, *ert* 28 is rather sparse-eared, although there is no doubt of its classification as an erectoides mutant. F_1 plants, heterozygous for two different alleles, are in general intermediary with regard to density.

Summarizing we conclude (1) that the most common morphological mutation type in barley, comprising the so-called erectoides mutants, arises after changes in several, although a restricted number of loci, (2) that in one locus eight independent cases of mutation have taken place; in others two to four; in ten out of 14 analysed loci only one mutation, and (3) that many, or most, mutations of one and the same locus manifest themselves by different phenotypical expressions; consequently, if no other linked mutations are at work, they are to be considered as different multiple alleles.

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INNEHÅLL.

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